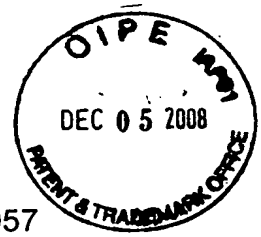
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re U.S. Patent Application

Jonathan S. STAMLER, et al.

Application Number: 10/508,957

Filed: February 3, 2005

Group Art Unit: 1609

Confirmation No.: 6780

Attny Dkt No.: STAM3022/ESS/PAD

Examiner: G. G. Huang

For: METHOD AND COMPOSITIONS BASED ON DISCOVERY OF METABOLISM OF NITROGLYCERIN

BRIEF ON APPEAL

MAY IT PLEASE YOUR HONORS:

i) REAL PARTY IN INTEREST

The real party in interest is Duke University of Durham, NC.

ii) RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences with respect to the claimed invention which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal known to appellant, appellant's legal representative or assignee.

iii) STATUS OF CLAIMS

Claims 75-84 are pending in this application. The present appeal is taken from the final rejection of claims 75-84.

Claims 1-74 have been cancelled from the application and are no longer pending.

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iv) STATUS OF AMENDMENTS

A request for reconsideration was filed on July 31, 2008. The Advisory Action of August 20, 2008 indicated that the request for reconsideration was considered but did not place the application in condition for allowance. Included with the Advisory Action was a translation of WEISCHER et al (DE 4420102), a reference relied upon by the Examiner throughout the prosecution of the application. The translation is relied upon in this Appeal Brief and cited in the Evidence Appendix.

v) SUMMARY OF CLAIMED SUBJECT MATTER

The inventors of the present application were the first to discover that the biotransformation of nitroglycerin occurs predominantly in mitochondria through a previously unknown reductase action of the known enzyme mitochondrial aldehyde dehydrogenase (mtALDH) and that attenuated biotransformation of nitroglycerin by mtALDH underlies nitrate tolerance (page 1, lines 5-10). With the discovery of this biotransformation, the inventors of the present invention have developed a method of activating inactivated mtALDH in a patient who has received nitroglycerin therapy and has become nitroglycerin tolerant so the patient no longer responds to nitroglycerin.

Claim 75 is a method of activating inactivated mtALDH in a patient who has received nitroglycerin therapy and has become nitroglycerin tolerant so the patient no longer responds to nitroglycerin (page 1, lines 5-10), comprising administering inactivated mtALDH activating effective amount of agent selected from the group consisting of dihydrolipoic acid, dithiothreitol and tris(2-carboxyethylphosphine) (page 1, last full paragraph; page 37, lines 1-19; Example XXXIII on page 45; and Figure 1).

Claim 84 recites a method for restoring clinical sensitivity to nitroglycerin to a patient who has lost sensitivity to nitroglycerin so that the patient no longer responds to nitroglycerin (page 3, lines 15-20), comprising administering to the patient a nitroglycerin sensitivity restoring amount of dihydrolipoic acid, dithiothreitol or tris(2-carboxyethylphosphine) (page 1, last full paragraph; page 37, lines 1-19; Example XXXIII on page 45; and Figure 1).

vi) GROUNDS OF REJECTION TO BE REVIEWED UPON APPEAL

The issues on appeal are as follows:

I. Whether Claims 75-84 satisfy the written description requirement pursuant to 35 U.S.C. 112, first paragraph;

II. Whether Claims 75-84 satisfy the enablement requirement pursuant to 35 U.S.C. 112, first paragraph;

III. Whether Claims 75-84 are definite under 35 U.S.C. 112, second paragraph;

IV. Whether Claims 75-78 and 81 are anticipated under 35 U.S.C. 102 by WEISCHER et al. (DE 4420 102A1);

V. Whether Claims 75-77, 79, and 82 are obvious under 35 U.S.C. 103 as being unpatentable over WEISCHER et al. (DE 4420 102 A1) in view of Pruijn et al. (Interplay between Vitamin E, Glutathione, and Dihydrolipoic Acid in Protection and Lipid Peroxidation); and

VI. Whether Claims 75-83 are obvious under 35 U.S.C. 103 as being unpatentable over WEISCHER et al. (DE 4420 102 A1), Pruijn et al. (Interplay between Vitamin E, Glutathione, and Dihydrolipoic Acid in Protection and Lipid Peroxidation), and Getz et al. (A Comparison between the Sulfhydryl Reductants

Tris(2-carboxyethyl)phosphine and Dithiothritol for Use in Protein Biochemcistry, analytical Biochemistry).

vii) ARGUMENT

I. CLAIMS 75-84 SATISFY THE WRITTEN DESCRIPTION REQUIREMENT PURSUANT TO 35 U.S.C. 112, FIRST PARAGRAPH

Appellants most respectfully submit that the Final Rejection fails to satisfy its burden in showing that the claims do not satisfy the written description requirement. The Patent Office has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed, *Wertheim*, 541 F.2d at 262, 191 USPQ at 96 (CCPA 1976).

The written description inquiry itself is a factual one and must be assessed on a case-by-case basis. *See Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1561, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991). The Guidelines for Examination of Patent Applications Under the 35 USC 112, paragraph 1 (Guidelines) provide that the examination of claims for compliance with the written description requirement should include:

i) a determination as to what the claim as a whole covers;

ii) a full review of the application to determine how the application provides support for the claimed invention including each recitation and/or step; and

iii) a determination as to whether one skilled in the art would recognize that the applicant was in possession of the claimed invention as a whole at the time of filing (See Fed. Reg. 66(4):1105).

The Final Rejection does not address any of these considerations. In this regard, Appellants respectfully submit that the rejection is improper as a matter of law.

Nevertheless, Appellants note that the Final Rejection contends that the disclosure does not support the type of patient being treated (i.e., a patient that no longer responds to nitroglycerin) in claims 75 and 84. Additionally, Claim 84 is rejected for reciting the phrase "nitroglycerin sensitivity restoring amount" (Final Rejection, page 4, second full paragraph).

While the specification may not explicitly provide for a patient that no longer responds to nitroglycerin, an invention need not be described *ipsis verbis* in the specification in order to satisfy the disclosure requirements. If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met. *Vas-Cath*, 935 F.2d at 1563, 19 USPQ2d at 1116; *Martin v. Johnson*, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972) (stating "the description need not be in *ipsis verbis* [i.e., "in the same words"] to be sufficient").

CLAIM 75

Claim 75 as a <u>whole</u> is directed to a method for activating inactivated mtALDH in a patient who has received nitroglycerin therapy and has become nitroglycerin tolerant so the patient no longer responds to nitroglycerin comprising administering inactivated mtALDH activating effective amount of an agent selected from the group consisting of dihydrolipoic acid, dithiothreitol and tris(2-carboxyethylphosphine). In other words, claim 75 is directed to treating a nitroglycerin tolerant patient with

agents that activate inactivated mtALDH. The patient is treated without reference to the disorder for which nitroglycerin was administered to cause tolerance.

A full review of the application shows that Appellants were in possession of the claimed invention at the time the application was field. At the outset, the specification makes it clear that the invention is directed to therapies for patients for whom nitroglycerin therapy administration is indicated (page, 1, lines 1-5).

The specification teaches at page 3, lines 15-20 that the nitroglycerin tolerance can be reversed. Thus, the specification does contemplate administering the recited compounds to a patient no longer responsive to nitroglycerin in an effort to reverse the tolerance.

The specification at page 11, line 20 to page 12, line 20 of the specification describes the phrase "capable of activating mtALDH" and provides dosages for the compounds recited in claims 75. Background Example 4 beginning on page 37 of the specification discusses dosages for the compounds recited in claims 75. The results of Background Example 4 are shown in Figure 1. Thus, a full review of the specification shows that specific dosages for DTT, DHLA and TCEP are provided and that that the specification does discuss reversing nitroglycerin tolerance or restoring nitroglycerin sensitivity in a patient not longer responds to nitroglycerin. One skilled in the art would consider these teachings as a whole conclude that the present disclosure satisfies the written description requirement.

Moreover, Examples XXXII and XXXIII on page 45 show restoring sensitivity to nitroglycerin in a patient. Thus, examples directed to the claimed invention are provided. The Final Rejection takes the position that these examples are insufficient as they are merely directed to treating angina. However, a full review of the application to determine how the application provides support for the claimed

invention is required. Indeed, there is no indication that one skilled in the art would not consider the additional teachings of the specification when reviewing these examples.

Upon considering the claims as a whole and reviewing the specification in full, one skilled in the art would recognize that applicant was in possession of the claimed invention as a whole at the time of filing.

Appellants respectfully request that this aspect of the rejection be reversed.

CLAIM 84

Claim 84 as a whole is directed to a method for restoring clinical sensitivity to nitroglycerin to a patient who has lost sensitivity to nitroglycerin so that the patient no longer responds to nitroglycerin.

Appellants respectfully submit that the type of patient being treated in the claim is supported for the same reasons noted above for Claim 75.

Moreover, the phrase "nitroglycerin sensitivity restoring amount" is supported at page 11, line 20 to page 12, line 20 of the specification. In addition, Background Example 4 beginning on page 37 of the specification discusses dosages for the compounds recited in claims 75 and 84. The results of Background Example 4 are shown in Figure 1. Thus, specific dosages for DTT, DHLA and TCEP are provided in the specification. Examples XXXII and XXXIII on page 45 show restoring sensitivity to nitroglycerin in a patient.

Appellants respectfully submit that the written description rejection is improper as a matter of law, and respectfully request that it be reversed.

II. CLAIMS 75-84 SATISFY THE ENABLEMENT REQUIREMENT PURSUANT TO 35 U.S.C. 112, FIRST PARAGRAPH

Appellants respectfully submit that claims 75-84 satisfy the enablement requirement.

The Final Rejection contends that the claim 75-84 fail to satisfy the enablement requirement because the disclosure does not reasonably provide enablement for "every" condition (See Final Rejection, page 4, third full paragraph).

Claim 75 recites a method for activating inactivated mtALDH in a patient who has received nitroglycerin therapy and has become nitroglycerin tolerant so the patient no longer responds to nitroglycerin. Claim 84 recites a method for restoring clinical sensitivity to nitroglycerin to a patient who has lost sensitivity to nitroglycerin so that the patient no longer responds to nitroglycerin. In other words, the claims require a patient who has received nitroglycerin therapy and has become nitroglycerin tolerant.

The invention is independent of the disease for which nitroglycerin is administered and is directed to restoring clinical sensitivity to nitroglycerin to a patient who has lost clinical sensitivity to nitroglycerin (see claims 75 and 84). A condition that would not be treated by nitroglycerin is not encompassed by the claims.

Thus, contrary to the position taken by the Final Rejection, claims 75 and 84 neither recite nor encompass "every" disorder.

The Final Rejection fails to provide any evidence that the claims are not enabled by the present disclosure. It is a well founded principle that any assertion by the Patent Office that the enabling disclosure is not commensurate in scope with the protection sought must be supported by the evidence or reasoning substantiating the doubt so expressed.

As a matter of law, the expressed teaching of the patent specification cannot be controverted by mere speculation and unsupported assertions on the part of the Patent Office. As stated by the Court of Customs and Patent Appeals in the case of *In re Dinh-Nguyen and Stanhagen*, 181 USPQ 46 (CCPA 1974):

> Any assertion by the Patent Office that the enabling disclosure is not commensurate in scope with the protection sought must be supported in evidence or reasoning substantiating the doubt so expressed. 181 USPQ at 47.

Such a standard must be applied with great care when the Examiner's conjecture is contrary to the teachings of the specification.

The Final Rejection cites to Kennedy, T., et al., JAMA 246(2) and the Physicians Desk reference in support of its position that nitroglycerin does not treat any "every" condition. While the documents discuss the general state of the art and show that one skilled in the art would know what conditions could be treated by nitroglycerin, none of the publications contradict any of the statements made in the specification or could be considered as evidence that the claimed invention is not enabled. In this regard, the publications are irrelevant towards determining whether the claims satisfy the enablement requirement.

At the top of page 7, the Final Rejection acknowledges that the specification does provide several examples that give guidance to one skilled in the art as how to practice the claimed invention. Rather, the Final Rejection states that these examples are deficient as they do not provide for all possible conditions for nitroglycerin treatment and tolerance.

However, Appellants respectfully submit that the specification plainly shows how to make and use the invention. At page 11, line 20 to page 12, line 20 of the specification, the phrase "capable of activating mtALDH" is recited and provides dosages for the compounds recited in claims 75 and 84. In addition, Background

Example 4 beginning on page 37 of the specification discusses dosages for the compounds recited in claims 75 and 84. Examples XXXII and XXXIII on page 45 exemplify dosages that might be used

As the Final Rejection fails to provide any evidence that the claims do not satisfy the enablement requirement, Appellants respectfully request that the rejection be reversed.

WRITTEN DESCRIPTION AND ENABLEMENT REJECTIONS IN GENERAL

It has been the experience of the undersigned that written description and enablement rejections are imposed on a "knee -jerk" basis without regard to the contents of the disclosure where specific diseases and particular compounds are not claimed. The case law does not support the use of these rejections. The reason is so that inventions can be commercialized. Indeed, what company would invest millions of dollars (as is often the case with inventions involving the life sciences) commercializing an invention, when another party can avoid the claims by brainstorming alternatives to the specifics that are claimed.

The result of these "knee-jerk" rejections is to unnecessarily delay prosecution and commercialization of an invention that may ameliorate the suffering of a patient , or even save lives. The delay resulting from these rejections could often be avoided by considering the disclosure as a whole and reviewing the relevant case law.

Appellants respectfully request that theses rejections be reversed.

III. CLAIMS 75-84 ARE DEFINITE TO ONE SKILLED IN THE ART

Appellants most respectfully submit that claims 75-84 are definite to one skilled in the art. MPEP § 2173.02 states that the essential inquiry pertaining to whether the claims satisfy the second paragraph of 35 U.S.C. 112 is whether the claims set out and circumscribe a particular subject matter with a reasonable degree

of clarity and particularity. Definiteness of claim language must be analyzed, not in a vacuum, but in light of:

(A) The content of the particular application disclosure;

(B) The teachings of the prior art; and

(C) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made.

The Final Rejection contends that the phrases "administering inactivated mtALDH activating effective amount of agent" and "a nitroglycerin sensitivity restoring amount" are indefinite (page 8, second pull paragraph and page 12, first full paragraph). At page 8, the Final Rejection states that "For purposes of examination, the administration of any amount of dihydrolipoic acid, dithiothreitol, or tris(2-carboxyethylphosphine) will be appropriate".

CLAIM 75

The specification at page 11, line 20 to page 12, line 20 of the specification describes the phrase "capable of activating mtALDH" and provides dosages for the compounds recited in claim 75. In addition, Background Example 4 beginning on page 37 of the specification discusses dosages for the compounds recited in claim 75. The results of Background Example 4 are shown in Figure 1. Examples XXXII and XXXIII on page 45 exemplify dosages that might be used. Thus, specific dosages for DTT, DHLA and TCEP are provided in the specification.

The Final Rejection acknowledges at the top of page 12 that the specification provides supports for a dosage range for the compounds recited in the claims. Rather, the Final Rejection states that "Applicant's argument with respect to the support for the dosage range in paragraph 59 is not persuasive as it is directed to a non-elected invention". The Final Rejection also states that "The argument for the

reference Example 4 and Figure 1 is not persuasive as it is in vitro data that does not reflect "administering inactivated mtALDH activating effective amount of agent".

One skilled in the art would not completely disregard the teachings of a disclosure because the patent Office has arbitrarily decided the claims of the application are directed to more than one invention. Indeed, Appellants respectfully submit that one skilled in the art would consider the disclosure as a whole. Moreover, while Background Example 4 provides in vitro data, there is no indication that one skilled in the art would disregard this experiment, or would not be able to gain some insight as to the amount of the compounds that need to be administered to obtain an "administering inactivated mtALDH activating effective amount of agent". In this regard, the content of the disclosure plainly supports the position that the claimed invention is definite to one skilled in the art.

Furthermore, Appellants submit that the phrase "administering inactivated mtALDH activating effective amount of agent" is somewhat analogous to the phrase "effective amount" for purposes of determining whether the phrase is definite to one skilled in the art. MPEP § 2173.05 (c) III provides that the proper test for determining whether the phrase "effective amount" is definite is whether or not one skilled in the art could determine specific values for the amount based on the disclosure. See *In re Mattison*, 509 F. 2d 563, 184 USPQ 484 (CCPA 1975). As noted above, the disclosure does provide guidance as to specific amounts in which to administer the recited compounds. Thus, the present application does allow one skilled in the art to determine specific values based on the disclosure.

MPEP § 2173.05(c)III also states that the phrase "an effective amount" has been held to be indefinite when the claims fails to state the "function" which is to be achieved and more than one effect can be implied from the specification or the

relevant art. *In re Fredericksen*, 213 F. 2d 547, 102 USPQ 35 (CCPA 1954). This is plainly not the case for the phrase "administering inactivated mtALDH activating effective amount of agent".

Appellants further note that the specification does discuss how to determine the activity of mtALDH. For example, at the bottom of page 27 a method of determining the effectiveness of a dose of nitrate on a patient is disclosed. This method comprises purifying mtALDH from a patient and determining the activity of the purified mtALDH to determine a dose that is effectively metabolized and does not cause inactivation of mtALDH. Thus, the specification does provide guidance as to administering an inactivated mtALDH activating effective amount of an agent.

In view of the above, Appellants respectfully submit that one skilled in the art would not interpret the phrases an "inactivated mtALDH activating effective amount" as encompassing any amount. The disclosure does describe particular dosages, and there is no evidence that suggests one skilled in the art would find this phrase indefinite. Claim 74 is definite to one skilled in the art.

CLAIM 84

As noted above, claim 84 is directed to a method for restoring clinical sensitivity to nitroglycerin to a patient who has lost sensitivity to nitroglycerin so that the patient no longer responds to nitroglycerin.

The present specification at page 11, line 20 to page 12, line 20 supports the phrase and provides dosages for the compounds recited in claim 84. In addition, Background Example 4 beginning on page 37 of the specification discusses dosages for the compounds recited in claims 75 and 84. The results of Background Example 4 are shown in Figure 1. Examples XXXII and XXXIII on page 45 also shows

restoring sensitivity to nitroglycerin sensitivity in a patient that no longer responds to nitroglycerin.

Appellants also submit that the phrase "a nitroglycerin sensitivity restoring amount" is analogous to the phrase "effective amount" for purposes of determining whether the phrase is definite to one skilled in the art. In this regard, claim 84 is definite for the same reasons as claim 75.

In view of the above, Appellants respectfully submit that one skilled in the art would not interpret the phrases an "a nitroglycerin sensitivity restoring amount" as encompassing any amount. The disclosure does describe particular dosages, and there is no evidence that suggests one skilled in the art would find these phrases indefinite. Thus, claim 84 is definite to one skilled in the art.

Appellants most respectfully submit that the claims are definite to one skilled in the art, and respectfully request that the rejection be reversed.

IV. CLAIMS 75-78 AND 81 ARE NOT ANTICIPATED UNDER 35 U.S.C. 102 BY WEISCHER ET AL.

WEISCHER fails to anticipate claims 75-78. To anticipate a claim, the reference must teach each and every recitation of the claim.

> A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). The identical invention must be shown in as complete detail as is contained in the ... claim. *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed Cir. 1989). The elements must be arranged as required by the claim, but this is not an *ipsissimis verbis* test, i.e., identity of terminology is not required. *In re Bond*, 910 F.2d 831, 15 USPQ2d 1566 (Fed.Cir. 1990).

Claim 75 recites a method for activating inactivated mtALDH in a patient who has received nitroglycerin therapy and has become nitroglycerin tolerant so the patient no longer responds to nitroglycerin. Claim 84 recites a method for restoring clinical sensitivity to nitroglycerin to a patient who has lost sensitivity to nitroglycerin so that the patient no longer responds to nitroglycerin. In other words, the claims require a patient who has received nitroglycerin therapy and has become nitroglycerin tolerant.

WEISCHER teaches a pharmaceutical combination preparation consisting of alpha-lipoic acid, dihydrolipoic acid and their oxidized or reduced R- or S-enantiomers and at least an organic nitrate, calcium- or ACE-inhibitor (WEISCHER, translation by Schreiber Translation, Inc., pg. 3, lines 1-10). WEISCHER describes that the combination preparation is based on a synergistic effect (See page 6, third full paragraph). For example, as indicated in the result section of WEISCHER, nitroglycerin plus alpha-lipoic acid gives a stronger effect than alpha-lipoic acid alone (page 7, line 15-20).

While WEISCHER may embrace administering a combination preparation to prevent nitroglycerin tolerance, WEISCHER never administers the combination preparation to a patient no longer responsive to nitroglycerin. Rather, WEISCHER administers the combination preparation with the hopes of preventing nitroglycerin tolerance. In this regard, WEISHCER makes every effort to prevent the patient from falling into a state wherein the patient is no longer responsive to nitroglycerin. WEISCHER provides no suggestion or reasonable expectation of success that one can reverse the effects of nitroglycerin tolerance in patient that no longer responds to nitroglycerin.

The Final Rejection acknowledges that WEISCHER does not explicitly disclose treating a patient that is no longer responsive to nitroglycerin. Rather, the Final Rejection contends that the patient treated by WEISCHER "will inherently have some degree of tolerance". Appellants respectfully disagree.

However, the fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (reversed rejection because inherency was based on what would result due to optimization of conditions, not what was necessarily present in the prior art); *In re Oelrich*, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981).

WEISCHER does not administer the combination preparation to a patient as claimed (i.e., no longer responsive to nitroglycerin). Indeed, as noted above, WEISCHER actually suggests that a combination preparation should be administered before tolerance occurs and certainly before a patient no longer responds to nitroglycerin. In this regard, the Final Rejection fails to show that WEISCHER necessarily administers the combination preparation to a patient that no longer responds to nitroglycerin.

Indeed, even if WEISCHER potentiates a response, potentiation does not mean restoring clinical sensitivity in the claimed milieu. For example, Appellants note that there are a number of articles that teach that N-acetylcysteine potentiates the effect of nitroglycerin, that is makes possible the use of less nitroglycerin for the same effect. See Loscalzo, J., J. Clin. Invest. 76(2), 703-708 (8/85) for *in vitro* effect, especially Figure 1 at page 704 which shows the potentiation effect; and Horowitz, M.B. et al., Circulation 68(6), 1247-1253 (12/83), wherein the abstract indicates *in vivo* potentiation effect; abstract of Nishikawa, Y., J. Cardiovasc. Pharmacul. 32, 21-

28 (1998); and abstract of Tates, Y., et al. Heart Vessels, 13(6), 263-268 (1998). Copies of Loscalzo, Horowitz, Nishikawa and Tate are attached and cited in the Evidence Appendix.

But despite potentiating the effect of nitroglycerin, N-acetylcysteine does not restore clinical sensitivity to nitroglycerin to a patient who has lost such sensitivity (no longer responds to nitroglycerin). See Figure 1 of the present application and Figure 4 of TCM 16(8), 259-265, copy attached. Thus, one skilled in the art can not necessarily predict how successful these compounds are in treating patients that are no longer responsive to nitroglycerin. In this regard, Appellants respectfully submit that the potentiation effect taught by WEISCHER does not provide a reasonable expectation of success for the sensitivity restoration effect recited in the claims.

In support of its position, the Final Rejection also states that WEISCHER claims the use nitroglycerin, and other nitrates with alpha-lipoic acid/dihydrolipoic acid in Claim 21. However, claims 21 does not refer to a particular patient group and does not disclose or suggest that the combined preparation was administered to a patient that has become nitroglycerin tolerant.

WEISCHER further discloses embodiments directed to treating ischemia (Translation, page 17, lines 10-30). In other words, WEISCHER discloses that the administration of the compounds can negate the presence of an insufficient amount of oxygen resulting from ischemia. In this regard, WEISCHER teaches away from the claims herein where administration is to activate an enzyme that has become oxidized. WEISCHER certainly does not discuss that the biotransformation of nitroglycerin occurs predominantly in mitochondria through a reductase action of the mtALDH and that attenuated biotransformation of nitroglycerin by mtALDH underlies nitrate tolerance, as discovered by the inventors for the first time.

Therefore, the claims are also unobvious over WEISCHER, and Appellants respectfully request that the rejection be reversed.

V. CLAIMS 75-83 ARE NOT OBVIOUS OVER WEISCHER AND PRUIJN.

WEISCHER fails to anticipate or render obvious claims 75-83 for the reasons noted above.

PRUIJN studies the relationship between vitamin E, glutathione and dihydrolipoic acid in protecting against lipid peroxidation.

PRUIJN does not disclose or suggest activating inactivated mtALDH, or even administering DTT to overcome nitroglycerine tolerance in a patient. In this regard, it would not have been obvious to one skilled in the art at the time of the invention to substitute DTT for dihydrolipoic acid. There is no suggestion that DTT is an activator of mtALDH so as to cause the conversion of nitroglycerin to 1,2- glyceryl dinitrate as provided for by claim 75.

In view of the above, PRUIJN fails to remedy the deficiencies of WEISCHER for reference purposes. Appellants respectfully submit that the proposed combination of WEISCHER in view of PRUIJN fails to disclose or suggest the claimed invention.

Thus, Appellants ask that the rejection be reversed.

VI. CLAIMS 75-83 ARE NOT OBVIOUS OVER WEISCHER, PRUIJN AND GETZ

The proposed combination of WEISCHER in view of PRUIJN fails to render obvious claims 75-83 for the reasons noted above.

GETZ compares the properties of DTT and TCEP (page 73, left column, lines 1-10). GETZ does not disclose or suggest activating inactivated mtALDH in a patient

x) RELATED PROCEEDINGS APPENDIX

None.

who has received nitroglycerin therapy and has become nitroglycerin tolerant so the patient no longer responds to nitroglycerin, by administering an inactivated mtALDH activating effective amount of an agent selected from the group consisting of dihydrolipoic acid, dithiothreitol and tris(2-carboxyethylphosphine). Indeed, GETZ does not even discuss activating inactivated mtALDH, or overcoming nitroglycerin tolerance in a patient.

Thus, GETZ fails to remedy the deficiencies of WEISCHER and PRUIJN for reference purposes.

Thus, Appellants ask that the rejection be reversed.

Conclusion

From the foregoing discussion, it is believed that the rejections of claims 75-84 are improper and should be reversed. Such action is accordingly respectfully requested.

A check is submitted with this brief on appeal for the fee set forth in 37 CFR 41.20(b)(3). Please charge any additional fees required to Deposit Account No. 02-200.

Respectfully submitted,
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viii) CLAIMS APPENDIX

75. A method of activating inactivated mtALDH in a patient who has received nitroglycerin therapy and has become nitroglycerin tolerant so the patient no longer responds to nitroglycerin comprising administering inactivated mtALDH activating effective amount of agent selected from the group consisting of dihydrolipoic acid, dithiothreitol and tris(2-carboxyethylphosphine).

76. The method of claim 75 where the patient is affected with a disorder selected from the group consisting of angina, restenosis, heart failure, portal hypertension, asthma and rectal spasm.

77. The method of claim 76 where the patient is affected with angina.

78. The method of claim 77 where the agent is dihydrolipoic acid.

79. The method of claim 77 where the agent is dithiothreitol.

80. The method of claim 77 where the agent is tris(2-carboxyethylphosphine).

81. The method of claim 75 where the agent is dihydrolipoic acid.

82. The method of claim 75 where the agent is dithiothreitol.

83. The method of claim 75 where the agent is tris(2-carboxyethylphosphine).

84. A method for restoring clinical sensitivity to nitroglycerin to a patient who has lost sensitivity to nitroglycerin so that the patient no longer responds to nitroglycerin comprising administering to the patient a nitroglycerin sensitivity restoring amount of dihydrolipoic acid, dithiothreitol or tris(2-carboxyethylphosphine).

ix) EVIDENCE APPENDIX

- A translation of WEISCHER et al. by the Schreiber Translation, Inc.
- Loscalzo, J., J. Clin. Invest. 76(2), 703-708 (8/85)
- Horowitz, M.B. et al., Circulation 68(6), 1247-1253 (12/83)
- Nishikawa, Y., J. Cardiovasc. Pharmacul. 32, 21-28 (1998)
- Tates, Y., et al. Heart Vessels, 13(6), 263-268 (1998).

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Potentiation of the cardiovascular effects of nitroglycerin by *N*-acetylcysteine

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ABSTRACT The biochemical basis of the mechanism of vasodilatation by nitroglycerin (NTG) has not been previously investigated in man. However, evidence from in vitro studies suggests that NTG induces activation of guanylate cyclase via a series of enzymatic reactions that are modulated by the availability of sulfhydryl groups. Cysteine appears to be particularly effective in potentiating guanylate cyclase activation by NTG. To determine whether hemodynamic responsiveness to NTG in man might be modulated by sulfhydryl availability, concentration-response curves for effects of intravenously infused NTG on mean arterial pressure (MAP) and mean pulmonary capillary wedge pressure (PCW) were obtained in 10 patients undergoing cardiac catheterization for investigation of chest pain. NTG infusion was repeated 10 min after the intravenous infusion of 100 mg/kg of the cysteine source *N*-acetylcysteine (NAC). NAC induced no significant hemodynamic effect, but after NAC infusion there was a significant reduction both in the NTG infusion rate associated with a 10% fall from control values in MAP (25.8 ± 8.3 to 9.3 ± 2.7 μg/min; $p < .01$) and in the infusion rate inducing a 30% reduction in PCW (13.6 ± 4.6 to 4.2 ± 1.6 μg/min; $p < .02$). In a control group of five patients who received no NAC, there was no significant change in responsiveness to NTG between infusions. It is concluded that NAC potentiates the vasodilator effects of NTG in man. This suggests that sulfhydryl availability and/or redox state may be determinants of in vivo responsiveness to NTG.
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NITROGLYCERIN (NTG) has been used as an antianginal agent for over 100 years.[1] Although the efficacy of NTG in the treatment of myocardial ischemia and in some clinical settings of congestive heart failure is now well established, controversy remains concerning its mechanism of action at both the hemodynamic and cellular levels.[2] Furthermore, the fundamental biochemical mechanisms of action of NTG are not well understood.

Needleman et al.[3] observed that incubation of rabbit aortic strips with the sulfhydryl alkylating agent ethacrynic acid led to a reduction in sensitivity to NTG. It was further suggested that tolerance to NTG could be induced by oxidation of sulfhydryl groups.[4] These observations have led to the suggestion that the vasodilator action of NTG is closely linked to the availability of critical SH groups in vascular smooth muscle.

More recent studies using isolated tissues have tended to support this postulated role of sulfhydryl groups in modulating responses to NTG. There is now evidence consistent with the view that NTG indirectly activates guanylate cyclase and that the vasodilator effects of NTG are mediated by increased intracellular concentrations of guanosine 3′, 5′-monophosphate (cyclic GMP).[5, 6] Although possible modulation of this process by sulfhydryl availability may occur at several points, available data suggest that S-nitrosothiol compounds, formed by interaction of NTG with tissue sulfhydryl, activate guanylate cyclase.[7, 8] Of a number of S-nitrosothiols tested, S-nitroso-cysteine was most effective in stimulating guanylate cyclase activation.[7]

Thus production of S-nitrosothiols by interaction of NTG with tissue sulfhydryl groups may be an essential step in the development of NTG-induced vasodilation. Variability in tissue sulfhydryl availability might also offer an explanation for the wide disparity in hemodynamic responsiveness to NTG at any particular plasma NTG concentration.[9] However, to date no studies have been carried out to determine whether availability of sulfhydryl, or particularly cysteine, modulate responsiveness to NTG in intact animals or man. The current-

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ly reported series of studies were carried out to test the hypothesis that this interaction may be operative in patients with ischemic heart disease.

Methods

Patients. The study population consisted of 15 patients (12 men, three women) undergoing diagnostic cardiac catheterization and coronary arteriography for evaluation of chest pain. Presence of unstable angina pectoris, hemodynamically significant stenosis of the left main coronary artery, significant valvular heart disease, requirement for nitrate administration during the routine component of cardiac catheterization, or previous adverse reactions to NTG or *N*-acetylcysteine (NAC) excluded patients from entry to the study.

Informed consent was obtained in writing before cardiac catheterization. Administration of long-acting nitrate preparations or cutaneously administered NTG was halted 12 to 24 hr before catheterization; other prophylactic antianginal agents were continued in unchanged dosages and sublingually administered NTG was used as required.

Protocol. Left and right heart catheterizations were performed from the femoral approach. Cardiac index was determined by the Fick method. After completion of the diagnostic procedures, a No. 7F balloon-tipped catheter was advanced into the pulmonary artery. Baseline levels of pulmonary arterial and pulmonary capillary wedge pressures were recorded, with determination of mean pressures over a 30 sec period to minimize errors due to respiratory variation. Femoral artery pressure was recorded via the side-arm of a femoral arterial sheath.[10]

After determination of baseline pressures, NTG infusion was initiated into a peripheral vein by means of a Model 2206 Harvard infusion pump and nonabsorbent tubing (McGaw Laboratories, Irvine, CA). The initial rate of NTG infusion was 1 μg/min. Effects on heart rate and on femoral arterial, pulmonary arterial, and pulmonary capillary wedge pressures were determined at 5 min intervals. Infusion rates of NTG were increased every 5 min, with successive rates of 1, 2.5, 5, 10, 25, and 50 μg/min. Infusion was terminated after any particular rate had induced a greater than 10% fall in mean arterial pressure (MAP) or a greater than 30% fall in mean pulmonary capillary wedge pressure ($\overline{PCW}$).

After a further 5 min, 10 patients (the NAC group) received an infusion of 100 mg/kg body weight of NAC in 200 ml of 5% dextrose via a peripheral vein over 15 min. After a further 10 min, infusion of NTG was repeated, with the same hemodynamic end points.

To assess the magnitude of spontaneous changes in responsiveness to NTG during the time required for this infusion protocol, a control group of five subjects receiving only 5% dextrose (200 ml) between the first and second NTG infusions was also studied.

Analysis of results. Differences in baseline parameters between patients in the NAC group and control subjects were assessed by the two-tailed unpaired Student's t test. Effects of NAC infusion (or 5% dextrose) on hemodynamic parameters between groups were assessed by the Wilcoxon rank-sum test.

Dose-response relationships for individual patients were determined after each NTG infusion by linear regression as illustrated in figures 1 and 2. The end points of NTG infusion rates inducing a 10% reduction in MAP and a 30% reduction in $\overline{PCW}$ were obtained from the regression lines. In two patients (Nos. 3 and 4 in the control group) in whom less than 20% reductions in $\overline{PCW}$ had occurred at NTG infusion rates that reduced MAP by greater than 10%, no attempt was made to determine the NTG infusion rate for the 30% $\overline{PCW}$ reduction end point, since this would have involved considerable extrapolation.

Statistical comparison of NTG infusion rates at hemodynamic end points within groups before and after infusion of NAC (or 5% dextrose) was performed by the Wilcoxon signed-rank test. The changes in infusion rates of NTG between the first and second NTG infusions were also compared between groups by means of the Wilcoxon rank-sum test. Comparison of infusion rates was performed by expressing the second NTG infusion rate as a percentage of the first NTG infusion rate.

Drugs. NTG was made up as a 50 μg/ml solution in glass bottles containing 5% dextrose by dilution from ampules (Tridil; American Critical Care). Connecting tubing used for transfer of NTG from the glass syringe used in conjunction with the Harvard infusion pump was minimally absorptive of NTG (Tridilset; McGaw Laboratories). NAC for intravenous administration (Parvolex; Duncan, Flockhart and Co., London) was diluted in 5% dextrose solution.

Results

Patients. Characteristics of the patients are summarized in table 1. There was no significant difference between the NAC patients and control subjects with respect to ages or weight; previous exposure to long-acting nitrates and β-adrenoceptor antagonists was similar in the two groups.

Findings at cardiac catheterization and baseline hemodynamic values are summarized in table 2. Two

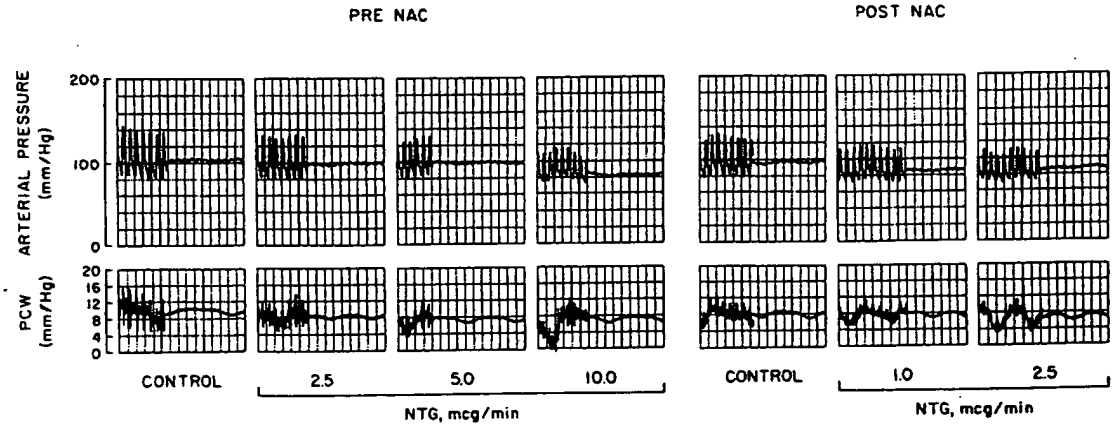

FIGURE 1. Effects of NTG on arterial and pulmonary capillary wedge phasic and mean pressures in one patient (No. 8, NAC group) before and after NAC.

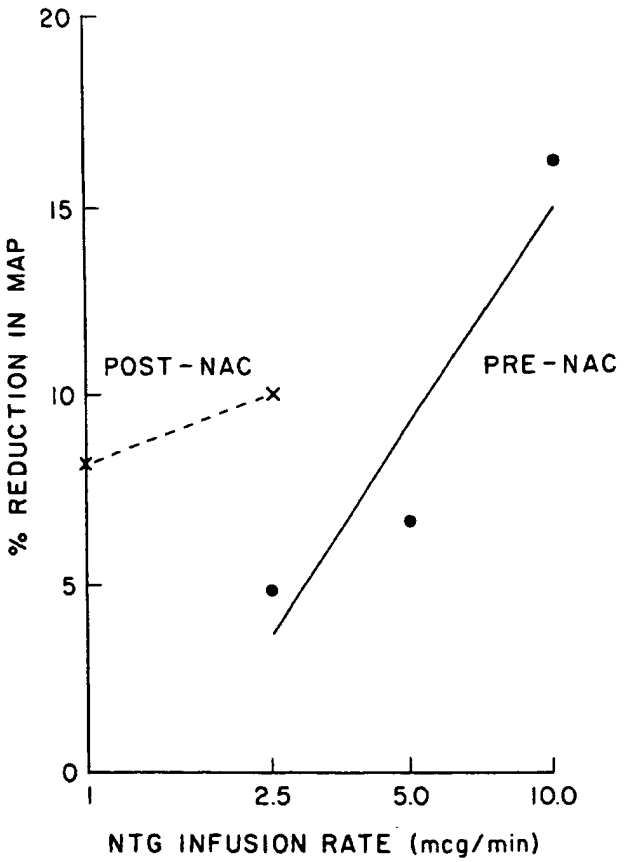

FIGURE 2. Regression lines for effects of NTG on MAP before and after NAC in the patient whose data are shown in figure 1.

subjects, one in each group, were found to have no hemodynamically significant coronary artery stenoses. In one patient only systemic artery pressure was measured. There was no significant difference between baseline values of MAP, mean pulmonary arterial, or $\overline{PCW}$ pressures, cardiac index, or heart rate between the NAC and control groups.

No patient had an initial MAP ≤75 mm Hg or an initial $\overline{PCW}$ ≤ 5 mm Hg, and the highest initial $\overline{PCW}$ was 17 mm Hg. Only one patient (No. 6, NAC group) had a left ventricular ejection fraction below 50%.

Initial response to NTG infusion. Threshold infusion rates of NTG inducing detectable falls in MAP or $\overline{PCW}$ varied considerably among the 10 NAC patients and five control subjects. The end points of a 10% reduction in systemic MAP or a 30% fall in $\overline{PCW}$ occurred at NTG infusion rates as low as 1.6 μg/min (table 3). In only one patient was the infusion rate associated with a 10% reduction in MAP greater than 50 μg NTG/min. However, two control patients (Nos. 3 and 4) developed less than 20% reductions in $\overline{PCW}$ at NTG infusion rates that reduced MAP by greater than 10%.

In the NAC group, mean maximal reduction in MAP was 11.1 ± 1.3%; maximal fall in $\overline{PCW}$ was 36.8 ± 4.3%. In the control group, mean maximal reduction in MAP was 8.3 ± 0.7%; maximal fall in $\overline{PCW}$ was 30.8 ± 3.2% (excluding patients 3 and 4). There was no significant difference in the NTG infusion rates initially producing a 10% reduction in MAP in the NAC and control groups. A Wilcoxon rank-sum test revealed no evidence of correlation between previous exposure of patients to long-acting nitrate preparations and initial sensitivity to infused NTG.

Effects of NAC or 5% dextrose infusion. No consistent hemodynamic effects were noted during infusion of either NAC or 5% dextrose in the NAC and control groups, respectively. Comparisons of hemodynamic parameters before the first and second NTG infusions (table 4) revealed no statistically significant fluctuations in baseline induced by NAC.

Effects of NAC on responsiveness to NTG. After infusion of NAC there was increased responsiveness to NTG, as determined by comparison of NTG infusion rates required to produce a 10% reduction of MAP or 30% reduction in $\overline{PCW}$ (table 3). Changes in responsiveness were determined separately for both of these hemodynamic end points (table 3). Because it appeared that changes in NTG responsiveness in the NAC group did not follow a Gaussian distribution,

TABLE 1
Patient characteristics

Patient No.	Age (yr)	Sex	Weight (kg)	Antianginal therapy (mg/day) β-Adrenoceptor antagonists	Antianginal therapy (mg/day) Nitrates	Mean NTG consumption (tablets/day)
NAC group						
1	43	M	45	M 300	I 680	5
2	54	M	67	M 300	I 200	2
3	59	M	87	M 100	—	<1
4	40	M	69	—	—	<1
5	56	M	76	P 160	N 13	<1
6	43	F	95	P 80	I 40	<1
7	43	M	95	P 240	I 80	<1
8	67	M	69	Na 40	I 40	<1
9	46	M	84	P 60	I 80	<1
10	60	M	72	—	—	<1
Mean	51.1		75.9			
SD	9.2		15.2			
Control group						
1	61	F	48	—	—	<1
2	44	F	67	P 640	I 640	2
3	57	M	117	P 160	I 80	2
4	35	M	122	P 240	I 120	1
5	61	M	85	P 160	I 40	<1
Mean	51.6		87.8			
SD	10.3		28.3			

P = propranolol; M = metoprolol; I = isosorbide dinitrate; N = NTG paste; Na = nadolol.

TABLE 2
Results of cardiac catheterization and baseline hemodynamics

Patient No.	No. of diseased vessels	LV ejection fraction (%)	Cardiac index (l/min/m²)	MAP	PA (mean)	$\overline{PCW}$	HR (bpm)
NAC group							
1	0	68	3.2	78	—	—	75
2	1	67	3.3	88	11	6	68
3	1	67	2.6	112	26	17	70
4	2	35	3.9	88	17	11	96
5	1	59	3.0	112	22	13	48
6	3	77	3.9	93	23	13	66
7	3	61	3.0	106	19	12	68
8	2	82	2.8	105	19	10	78
9	2	60	2.8	96	18	10	66
10	1	65	2.9	114	17	8	72
Mean		63	3.1	99.2	19.1	10.9	70.7
SD		13.3	0.3	12.3	4.4	3.5	12.0
Control group							
1	0	70	3.1	94	15	7	102
2	1	76	3.1	135	15	8	84
3	3	57	2.9	94	23	16	75
4	2	70	4.5	119	27	17	68
5	1	72	2.9	101	18	9	63
Mean		69	3.3	108.6	19.6	11.4	78.4
SD		7.1	0.7	17.8	5.4	4.7	15.4

LV = left ventricular; EF = ejection fraction; PA = pulmonary arterial pressure; HR = heart rate.

Wilcoxon rank-sum tests were used to compare these changes in the NAC and control groups.

There was a statistically significant increase in NTG responsiveness in the NAC group relative to the control group for both hemodynamic end points ($p < .01$ for MAP; $p < .05$ for $\overline{PCW}$). There was a significant correlation between the change in responsiveness as determined by comparison of NTG infusion rates inducing a 10% reduction in MAP (I MAP 10) and the initial I MAP 10 values ($r = .85$; $p < .01$); that is, the increase in hypotensive potency of NTG after NAC was most marked in patients who were initially least responsive to NTG. There was a far weaker correlation between initial NTG responsiveness measured by reduction in $\overline{PCW}$ and increase in responsiveness after NAC ($r = .32$; $p > .05$). There was no significant correlation between increase in responsiveness after NAC and previous treatment with long-acting nitrate preparations.

Within the control group, responsiveness to NTG varied moderately between the first and second infusions. However there was no consistent trend, with three patients showing a reduction and two patients a mild increase in responsiveness (table 3), suggesting that significant hemodynamic tolerance to NTG was not induced by the first infusion.

Comparison of changes in responsiveness to NTG between the NAC and control groups indicated a statistically significant ($p < .05$) difference between the two groups for both the MAP and $\overline{PCW}$ end points.

Adverse effects. NTG was well tolerated by most patients. However, one patient (No. 1, control group) experienced mild headache during infusion at maximum rate. One patient (No. 1, NAC group) developed transient cough and production of mucus 20 min after termination of the NAC infusion.

Discussion

The results of these experiments demonstrate that NAC potentiates hemodynamic responsiveness to NTG, as measured by changes in MAP and $\overline{PCW}$ per unit NTG infusion rate. This potentiation contrasts with the lack of spontaneous variability in responses to NTG in the control group. A number of possible mechanisms for the effects of NAC are revealed from consideration of the results of in vitro experiments related to the mechanisms of action of NTG.

The initially proposed model of the NTG "receptor"[4] was based on studies in preparations of rabbit aortic strips which indicated that specific tolerance to nitrates could be induced by prolonged exposure to NTG, potentiated by alkaline pH, but reversed by the reducing agent dithiothreitol. It was suggested that NTG (and organic nitrates) reversibly oxidizes one or more sulfhydryl groups in the "receptor," leading to the formation of a disulfide form of the receptor that has a much lower affinity for NTG. The inhibitory effects of ethacrynic acid, which alkylates sulfhydryl groups, on responsiveness to NTG in vitro provided further support for this hypothesis.[3]

The postulated role of cyclic GMP as an intracellular mediator of NTG-induced vasodilatation[5,6] makes the potential mechanism of modulation of NTG sensitivity more complex. First, a number of compounds have been shown to be potent activators of guanylate cyclase in arterial smooth muscle. These include several S-nitrosothiols, which would be formed when NO_2^- or nitric oxide react with a tissue sulfhydryl source.[7] In these experiments, it was found that cysteine was far more effective in stimulating activation of guanylate cyclase by NTG than other sulfhydryl-containing materials such as dithiothreitol, penicillamine, and reduced glutathione. Ascorbate was totally ineffective. This suggests that the interaction between cysteine and NTG is not purely a matter of increased availability of sulfhydryl groups or of alteration in tissue redox state.

TABLE 3
NTG infusion rates in NAC and control groups required to achieve hemodynamic end point

Patient No.	First NTG infusion		Second NTG infusion		Change in infusion rate (%)^	
	I MAP 10	I PCW 30	I MAP 10	I PCW 30	MAP	PCW
NAC group						
1	22.4	—	6.8	—	30.3	—
2	46.5	47.9	17.2	7.5	37.0	15.7
3	90.3	7.9	9.8	5.1	10.9	64.6
4	4.4	5.6	3.3	2.9	75.0	51.8
5	20.9	15.6	30.1	1.0	144.0	6.4
6	32.5	6.5	5.9	1.3	18.2	20.0
7	13.8	5.3	4.3	6.6	31.2	124.5
8	5.5	16.3	2.2	22.4	40.0	137.4
9	9.6	2.6	6.5	1.1	67.7	42.3
10	12.3	14.3	7.4	3.0	60.2	42.9
Mean	25.8	13.6	9.3	4.2		
SD	26.2	14.5	8.5	5.1		
Control group						
1	34.7	25.7	29.5	17.4	85.0	67.7
2	33.9	34.7	23.4	27.5	69.0	79.3
3	33.9	—	53.7	—	158.4	—
4	26.9	—	42.7	—	158.7	—
5	5.0	1.6	8.4	3.5	168.0	218.8
Mean	27.0	20.7	31.5	16.1		
SD	12.5	22.3	17.4	15.6		

I MAP 10 = NTG infusion rate (μg/min) inducing 10% fall in MAP; I PCW 30 = NTG infusion rate (μg/min) inducing 30% fall in mean PCW.

^Percent of first infusion required to achieve hemodynamic end point during second infusion.

Further modulation of NTG responsiveness by variation in sulfhydryl availability is possible at least at two other points. NTG inactivation, catalyzed by the hepatic enzyme organic nitrate ester reductase, induces oxidation of glutathione; depletion of hepatic reduced glutathione can be demonstrated in vitro after prolonged NTG infusion.[11] Inhibition of hepatic organic nitrate ester reductase activity may produce prolongation of the plasma half-life of NTG.[12]

A final mechanism of sulfhydryl modulation of NTG responsiveness is apparent from the finding that hepatic[13] and pulmonary[14] guanylate cyclase is rapidly inactivated by molecular oxygen, sulfhydryl oxidants, or thiol alkylating agents and that the stimulation of guanylate cyclase activity by nitric oxide and S-nitrosocysteine is prevented by sulfhydryl oxidants. This suggests that sulfhydryl groups on the guanylate cyclase catalytic site are critical in its activation. Thus guanylate cyclase activity is likely to be susceptible to variations in tissue redox state.

The study reported here was performed in an attempt to evaluate, in a clinical setting, some of the issues raised from these in vitro studies. We chose to investigate the effects of short-term administration of NAC on hemodynamic response to NTG in patients with ischemic heart disease. NAC was chosen as the sulfhydryl source for a number of reasons. It was possible that a cysteine-containing material would produce optimal potentiation of responses to NTG.[7] NAC is extensively hydrolyzed to cysteine in vivo,[15] although plasma concentrations of unchanged NAC become detectable after long-term administration.[16] Furthermore, NAC has proved to be safe and rapidly effective when administered intravenously as a cysteine source in the emergency treatment of acetaminophen overdose.[17] We used a commercially available sterile preparation of NAC for intravenous administration (Parvolex),

TABLE 4
Mean values of hemodynamic parameters before the first and second NTG infusions

Group	MAP		$\overline{PCW}$		PA		HR	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
NAC								
Mean	99.2	101.1	10.9	11.1	19.1	19.0	70.7	70.1
SD	12.3	11.7	3.5	3.5	4.4	4.7	12.0	11.7
Control								
Mean	108.6	105.4	11.4	10.8	19.6	18.2	78.4	76.8
SD	17.8	19.2	4.7	5.4	5.4	6.2	15.4	13.6

which was infused at a rate similar to that used by Prescott et al.[17] The lack of significant hemodynamic effects of NAC in our study was in accordance with previous experience with the drug.

In view of the potential risk of inducing substantial decreases in MAP, the major end points chosen to assess patient responsiveness to NTG were the infusion rates that produced a 10% reduction in MAP and a 30% reduction in $\overline{PCW}$. However, it must be recognized that the hemodynamic end points chosen are representative only of the lower end of the NTG dose-response curve and that assessment of the extent of fluctuations in responsiveness to NTG cannot be extrapolated beyond the chosen end points.

Although each patient who received NAC served as his or her own control, a small control group of patients who did not receive NAC was included because of the possibility that changes in sensitivity to NTG could occur between infusions as a result of absorption of NTG onto infusion tubing, onset of true hemodynamic tolerance or potentiation, or changes in baseline values induced by infusion of 5% dextrose between NTG infusions. Although minor fluctuations in responsiveness to NTG were observed in the control group, there was no major change between infusions. This, together with the initial responsiveness of the NAC group to NTG infusion rates of less than 100 μg/min, suggests that the infusion system used was effective in minimizing losses of NTG.

Administration of NAC induced a clear-cut potentiation of hemodynamic responsiveness to NTG, assessed both on the basis of changes in MAP and $\overline{PCW}$. The hypotensive effects of NTG were more markedly increased in those patients who initially had been least responsive to NTG. The most probable explanation of these findings is that NAC potentiated the vasodilator effects of NTG, presumably in both arteries and veins. Although this cannot be stated with certainty in the absence of more extensive assessment of hemodynamic changes, the only other explanation (attenuation of the effects of NTG on cardiac output) seems unlikely.

It is possible that the greater potentiation after NAC of NTG effects in patients who were initially less responsive to the drug reflects variations among patients in initial availability of sulfhydryl groups and/or in tissue redox state. Although, the present study was not designed to evaluate this possibility, no evidence was found of effects of previous administration of long-acting nitrates on either initial responsiveness or the degree of NAC-induced potentiation. Thus, while tolerance to NTG may under some circumstances be induced by long-acting nitrates,[18] this did not appear to be the major determinant of responsiveness in the present series.

The results of our study suggest that, particularly in initially insensitive patients, enhanced hemodynamic responsiveness to NTG may be induced after NAC. This finding is in accordance with the results of previous in vitro studies. Whether this altered responsiveness is of clinical value in the management of ischemic heart disease and/or congestive heart failure remains to be addressed in future studies.

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Links

Effects of N-acetylcysteine on nitroglycerin-induced relaxation and protein phosphorylation of porcine coronary arteries.

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We investigated the effects of the sulfhydryl-donor, N-acetylcysteine (NAC), on nitroglycerin (NTG)-induced relaxation of the vascular smooth muscle. Addition of histamine to isolated porcine coronary arteries induced an initial rapid contraction followed by a gradual decrease in tonic contraction. NTG applied to the coronary artery strips before histamine caused relaxation of the histamine-induced rapid (3 min) and tonic (48 min) contraction. The inhibition of the tonic contraction by NTG was less at 48 min than at 3 min. Application of NAC (NTG-NAC) enhanced the relaxing effects of NTG on the histamine-induced tonic contraction rather than the acute contraction. In phosphorylation studies, changes in the phosphorylation of an intermediate filament, desmin, were parallel with changes in contraction in NTG-treated and NTG-NAC samples at 48 min. These phosphorylation changes of desmin at 48 min, which might be responsible for tonic phase contraction, were more extensive than those of myosin light chain (MLC) phosphorylation at 3 min, which might be responsible for acute contraction. These results suggest that treatment with the sulfhydryl donor, NAC, inhibited the phosphorylation of desmin associated with the enhancement of NTG-induced relaxation, which might be related to the mechanisms of recovery from NTG tolerance by sulfhydryl groups.

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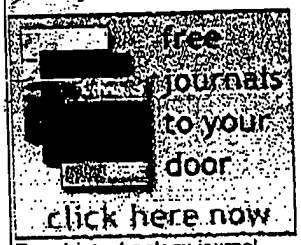

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Differential effects of N-acetylcysteine on nitroglycerin- and nicorandil-induced vasodila

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We investigated the role of the availability of sulfhydryl groups during vasodilation of the human coronary circulation induced by nitroglycerin and nicorandil. In patients with normal coronary arteries (n = 29) or with coronary artery disease (CAD; n = 26), coronary blood flow (CBF) and epicardial coronary artery diameter after intracoronary administration of 50 microg nitroglycerin or 0.5 mg nicorandil were measured, before and after the intravenous infusion of saline or 100 mg/kg of N-acetylcysteine (NAC). In normal subjects, saline infusion did not alter the nitroglycerin- and nicorandil-induced vasodilation in large epicardial coronary artery. In contrast, NAC potentiated both nitroglycerin- and nicorandil-induced vasodilation. In patients with CAD, nitroglycerin and nicorandil induced less dilation than in normal subjects. NAC augmented the nitroglycerin- and nicorandil-induced vasodilation in the small epicardial coronary artery, but not in the large epicardial segments. In both groups, NAC potentiated the increase in CBF in response to nitroglycerin. However, NAC had no effects on the CBF response to nicorandil. Sulfhydryl availability is at least one determinant of the in vivo responsiveness to nitroglycerin of conductance and resistance vessels in normal human coronary circulation. In patients with CAD, external augmentation of sulfhydryl availability did not affect the depressed response to nitroglycerin in the large epicardial coronary artery. Although nicorandil acts as an NO donor, similar to nitroglycerin, in dilating the epicardial coronary artery, other effects, such as the opening of K(ATP) channel, play a more important role in the nicorandil-induced vasodilation of resistance vessels.

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N-Acetylcysteine Potentiates Inhibition of Platelet Aggregation by Nitroglycerin

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Abstract

Platelet aggregation is currently felt to play an important role in the pathogenesis of ischemic vascular disorders. The smooth muscle relaxant, nitroglycerin, has been shown to inhibit platelet aggregation in vitro, but at concentrations that were felt to be unattainable in vivo. Because the in vivo action of nitroglycerin on smooth muscle cells has been shown to depend on the presence of reduced cytosolic sulfhydryl groups, the inhibitory effect of nitroglycerin on platelet aggregation was examined in the presence of the reduced thiol, *N*-acetylcysteine. Millimolar concentrations of *N*-acetylcysteine potentiated markedly the inhibitory effect of nitroglycerin on platelet aggregation induced by ADP, epinephrine, collagen, and arachidonate, decreasing the 50% inhibitory concentration (IC_{50}) ~50-fold for each of these agents. Other guanylate cyclase activators inhibited ADP-induced aggregation similarly and this inhibition was likewise potentiated by *N*-acetylcysteine. Platelet guanosine 3',5'-cyclic monophosphate content increased fivefold in the presence of nitroglycerin and *N*-acetylcysteine 2 min before maximal inhibition of ADP-induced aggregation was achieved, while simultaneously measured cyclic AMP did not change relative to base-line levels. In the absence of *N*-acetylcysteine, nitroglycerin induced a marked decrease in platelet-reduced glutathione content as *S*-nitroso-thiol adducts were produced. The synthetic *S*-nitroso-thiol, *S*-nitroso-*N*-acetylcysteine, markedly inhibited platelet aggregation with an IC_{50} of 6 nM. These data show that *N*-acetylcysteine markedly potentiates the inhibition of platelet aggregation by nitroglycerin and likely does so by inducing the formation of an *S*-nitroso-thiol adduct(s), which activate guanylate cyclase.

Introduction

The importance of platelets in the pathogenesis of myocardial ischemia and infarction has recently been recognized. Circulating platelet aggregates are more prevalent in patients with sudden coronary death than in patients who die of other causes (1, 2). Vasoactive substances released by platelets at sites of endovascular injury, such as thromboxane A_2 and lipoxygenase products, produce coronary vasospasm (3–5). Furthermore, upon platelet activation, the platelet membrane itself serves as a catalytic surface on which coagulation factors interact in the formation of fibrin (6), the presence of which further limits vessel patency.

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Organic nitrate vasodilators, particularly nitroglycerin, have been used for many years in the treatment of ischemic heart disease (7). The mechanism of action of these agents remains incompletely defined (8, 9), but very likely involves direct smooth muscle relaxation in conductive (coronary) arteries (10, 11). Given the role of the platelet in the pathophysiology of coronary ischemia, several groups have investigated the effect of organic nitrate vasodilators on in vitro platelet function (12–14). Agonist-induced aggregation was uniformly inhibited by nitrates in these early studies, but the concentration of nitrates required was quite high and not achievable pharmacologically, thus calling into question the therapeutic relevance of this observation.

The work of Needleman et al. (15) demonstrated that the vasodilator action of nitroglycerin depends critically on the availability of certain essential sulfhydryl groups in vascular smooth muscle cells. These in vitro observations were extended in vivo by Horowitz et al. (16), who showed that pretreating patients with the sulfhydryl agent, *N*-acetylcysteine, reduced the amount of nitroglycerin required to achieve a targeted reduction in mean arterial and mean pulmonary capillary wedge pressures.

Because of the importance of platelets in the pathogenesis of myocardial ischemia, the ability of organic nitrates to inhibit platelet function in vitro (albeit at excessive concentrations), and the potentiating effect of sulfhydryl agents on nitroglycerin action in smooth muscle cells, we investigated the effect of *N*-acetylcysteine on the inhibition of platelet aggregation by nitroglycerin and other organic nitrates. The data presented show the following: (*a*) that *N*-acetylcysteine markedly potentiates the inhibitory action of nitroglycerin on platelet aggregation in vitro to a variety of agonists; (*b*) that nitroglycerin (as other nitrate vasodilators) inhibit platelet aggregation by increasing intracellular cyclic GMP (cGMP) and *N*-acetylcysteine markedly potentiates this effect; (*c*) that nitroglycerin inhibits platelet aggregation through the formation of *S*-nitrosothiols, these latter compounds being both potent activators of guanylate cyclase and extremely potent inhibitors of platelet aggregation; and (*d*) that pharmacologically achievable concentrations of nitroglycerin in vivo may act to inhibit platelet aggregation, provided that there are sufficient reduced sulfhydryl groups present in the platelet, and that, therefore, an important therapeutic action of nitroglycerin may well be inhibition of platelet activation.

Methods

Materials. Epinephrine, ADP, sodium azide, and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB)[1] were purchased from Sigma Chemical Co. (St. Louis, MO). Calf skin collagen was obtained from Worthington Biochemical (Freehold, NJ). Sodium arachidonate was purchased from

1. *Abbreviations used in this paper:* DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); IC_{50}, inhibitory concentration 50%; PRP, platelet-rich plasma.

NuCheck-Prep (Elysian, MN). Metaphosphoric acid, sulfanilamide, and *N*-(1-naphthyl)ethylenediamine dihydrochloride were purchased from Aldrich Chemical Co. (Milwaukee, WI). Nitroglycerin was obtained from Marion Laboratories, Inc. (Kansas City, MO). Sodium nitroprusside was purchased from Abbott Laboratories (Chicago, IL). *N*-Acetylcysteine was purchased from Mead Johnson and Co. (Evansville, IL) and from Duncan, Flockhart, and Co., (London, England). Reduced glutathione (GSH) was purchased from Calbiochem (LaJolla, CA). Radioimmunoassay kits for the determination of cGMP and cyclic AMP (cAMP) were purchased from New England Nuclear (Boston, MA). All other materials were reagent grade or better. Deionized water was used throughout.

Platelets. Venous blood was obtained within 1 h of use from volunteers who had not ingested acetylsalicylic acid for at least 10 d and was anticoagulated with 13 mM sodium citrate. The platelet-rich plasma (PRP) was prepared by centrifugation at 160 *g* for 10 min. Platelet counts were determined with a Coulter counter (model F; Coulter Electronics, Inc., Hialeah, FL).

Platelet aggregation. Platelet aggregation was monitored using a standard nephelometric technique (17) in which 0.4-ml aliquots of PRP were incubated at 37°C and stirred at 900 rpm in a Payton dual-channel aggregometer (Payton Associates, Inc., Buffalo, NY). Aggregation was induced by addition of 11 μM ADP, 12.5 μM epinephrine, 0.12 mg/ml calf skin collagen, or 0.44 mM arachidonate and changes in light transmittance recorded using an Omniscribe recorder (Houston Instruments, Austin, TX). PRP was preincubated at 37°C for 4 min with *N*-acetylcysteine or with nitroglycerin, or first with *N*-acetylcysteine and then nitroglycerin, and sodium nitroprusside, sodium azide, sodium nitrite, or *S*-nitroso-*N*-acetylcysteine for 1 min before addition of agonist. Aggregation was quantitated by measuring either the extent of change of light transmittance or the maximal rate of change of light transmittance; the extent of change in transmittance was used in experiments comparing effects of nitroglycerin or other nitrates on aggregation induced by different agonists, while the rate of change in transmittance was used in experiments addressing the time course of inhibition of aggregation.

Cyclic nucleotide assays. Measurements of cGMP and cAMP were performed by radioimmunoassay. After incubating PRP at 37°C with nitroglycerin and/or *N*-acetylcysteine, the platelets were processed as described previously (14) and radioimmunoassays for cGMP and cAMP performed. Acetylation of samples with acetic anhydride was used to increase the sensitivity of the assays.

Platelet glutathione measurements. GSH was measured in platelets by the method of Beutler et al. (18), with slight modifications. At various times after incubation with nitroglycerin, PRP was treated with 1.67% glacial metaphosphoric acid, 0.02% disodium (ethylenedinitrilo)tetraacetate, and 3% NaCl. The mixture was centrifuged at 8,700 *g* for 4 min at 4°C and the supernatant neutralized with 1.0 M Na_2HPO_4. DTNB was used to detect free sulfhydryl groups spectrophotometrically (19, 20), using a molar extinction coefficient of 14,200 $M^{-1}cm^{-1}$ for the nitrothiophenolate ion (21).

Nitrite determination. Free nitrite was assayed in the supernatant of trichloroacetic acid extracts of platelets incubated with nitroglycerin with or without *N*-acetylcysteine by the method of Snell and Snell (22), which involved diazotization of sulfanilic acid and subsequent coupling with the chromophore *N*-(1-naphthyl)ethylenediamine. *S*-Nitroso-thiols were detected by assaying free nitrite by diazotization of sulfanilic acid in the presence and absence of 0.15% $HgCl_2$, the latter reagent catalyzing the hydrolysis of *S*-nitroso bonds (23).

Preparation of S-nitroso-N-acetylcysteine. *S*-Nitroso-*N*-acetylcysteine was prepared at 25°C by reacting equimolar concentrations of *N*-acetylcysteine with $NaNO_2$ at acidic pH (24, 25). Solutions turned from clear to rose-colored upon completion of the reaction. The *S*-nitroso-thiol was identified by visible absorption spectroscopy, having an absorption maximum of 550 nm. The completeness of the reaction was verified by measuring free nitrite in the presence and absence of 0.15% $HgCl_2$, as described in the above paragraph, and by measuring reduced sulfhydryl groups using DTNB. Due to the instability of the *S*-nitroso-derivatives, *S*-nitroso-*N*-acetylcysteine was prepared within 1 h of use, kept in acidic solution at 4°C, and diluted as necessary into aqueous buffer immediately before addition to assay systems.

Results

Inhibition of platelet aggregation by nitroglycerin. Nitroglycerin inhibited platelet aggregation induced by 11 μM ADP in a typical dose-response fashion (Fig. 1). The IC_{50} for this inhibition was 42 μM. Other agonists, including 12.5 μM epinephrine, 0.12 mg/ml calf skin collagen, and 0.44 mM arachidonate, were also inhibited with similar IC_{50}s (Table I). For each agonist, the extent of aggregation at any concentration of nitroglycerin was normalized to that in the absence of nitroglycerin and *N*-acetylcysteine.

Potentiation of the inhibitory effect of nitroglycerin by N-acetylcysteine. *N*-Acetylcysteine at 5.5 mM added to PRP 4 min before the addition of nitroglycerin (and 5 min before the addition of agonist) markedly potentiated the inhibitory action of nitroglycerin for each agonist tested (Fig. 1 and Table I). The IC_{50} shifted from ~42 to 0.71 μM for ADP with similar shifts noted for collagen, epinephrine, and arachidonate (Table I). At the concentration used in these experiments, *N*-acetylcysteine alone did not inhibit platelet aggregation; however, at higher concentrations, inhibition of aggregation by *N*-acetylcysteine alone was apparent. Lower concentrations of *N*-acetylcysteine also potentiated inhibition by nitroglycerin, the effect decreasing with decreasing concentrations of *N*-acetylcysteine.

Inhibitory effect of other guanylate cyclase activators on

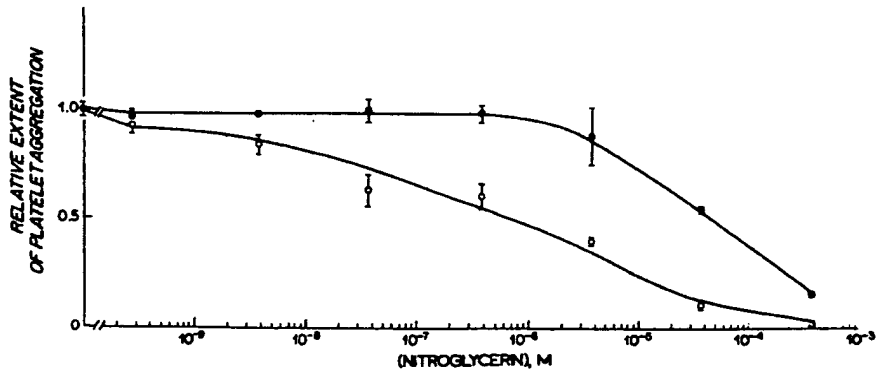

Figure 1. Potentiation of inhibition by nitroglycerin of ADP-induced platelet aggregation with *N*-acetylcysteine. Platelets in PRP were incubated at 37°C with (○) or without (●) 5.5 mM *N*-acetylcysteine for 4 min, then with various concentrations of nitroglycerin for 1 additional min, after which 11 μM ADP was added to initiate aggregation. Values on the ordinate reflect extent of aggregation relative to that in the absence of nitroglycerin and *N*-acetylcysteine (corresponding to 85% maximal light transmittance). Each point represents the mean±SEM of three experiments done in duplicate.

*Table I. Inhibition of Platelet Aggregation by Nitroglycerin and Potentiation of Inhibition by N-Acetylcysteine**

Agonist	IC_{50}	
	−*N*-Acetylcysteine	+*N*-Acetylcysteine
	μM	*μM*
ADP (11 μM)	42±2	0.71±0.10
Epinephrine (12.5 μM)	34±4	0.20±0.08
Collagen (0.12 mg/ml)	36±4	0.60±0.15
Arachidonate (0.44 mM)	45±6	1.00±0.10

* Aggregation of platelets in PRP was induced by addition of particular agonists after incubating platelets at 37°C with various concentrations of nitroglycerin ±5.5 mM *N*-acetylcysteine for 5 min. IC_{50}s each represent the mean±SEM for three experiments done in duplicate. For further details, see Methods.

ADP-induced platelet aggregation and potentiation by N-acetylcysteine. Nitroglycerin is a potent activator of guanylate cyclase (26) and, given that this activation requires the presence of reduced thiol groups (26), other guanylate cyclase activators were tested for their ability to inhibit ADP-induced platelet aggregation in the presence and absence of *N*-acetylcysteine. In Table II are listed the IC_{50}s for sodium acide, sodium nitroprusside, sodium nitrate, and nitroglycerin in the presence and absence of 5.5 mM *N*-acetylcysteine. These data show that while the IC_{50}s ranged widely (1.3 μM for sodium azide to 59,000 μM for sodium nitrite), in each case *N*-acetylcysteine potentiated the inhibition of ADP-induced aggregation, reducing the IC_{50}, on average, 50-fold (ranging from 22- to 87-fold for nitroprusside to nitrite, respectively).

Increase in platelet cGMP by nitroglycerin and potentiation by N-acetylcysteine. Mellion et al. (27) showed that sodium nitroprusside and nitric oxide inhibited ADP-induced platelet aggregation and that this inhibition was preceded by an early, large, and partly transient increase in intracellular cGMP. For this reason, the effect of nitroglycerin on platelet cGMP was assessed and the influence of *N*-acetylcysteine on this effect examined. Total platelet cGMP was measured by radioimmunoassay in quiescent platelets and platelets incubated with nitroglycerin with and without *N*-acetylcysteine. Platelets in PRP were incubated with various concentrations of nitroglycerin with or without 5.5 mM *N*-acetylcysteine for 5 min at 37°C, after which the platelets were processed as described previously (14) and cGMP determined. Table III lists the values obtained and indicates that nitroglycerin increased resting cGMP from base-line levels fourfold without *N*-acetylcysteine (from 0.20±0.04 to 0.80±0.04 pmol/10^8 platelets) and up to 19-fold with *N*-acetylcysteine (to 4.08±0.26 pmol/10^8 platelets) at the highest nitroglycerin concentrations used (390 μM).

The incubation time used in this experiment was chosen because inhibition by nitroglycerin of platelet aggregation in the presence of *N*-acetylcysteine is maximal by 4 min and because subsequent experiments evaluating the time course of changes in platelet aggregation were carried out for as long as 5 min after addition of reactants (see below). As had been shown previously for nitroglycerin (14), no change in platelet cAMP from basal levels of 3.5±0.5 pmol/10^8 platelets occurred in these experiments.

Time course of increase in platelet cGMP and inhibition of platelet aggregation. The temporal relationship between the increase in platelet cGMP and the decrease in ADP-induced platelet aggregation is shown in Fig. 2. Platelets in PRP were incubated at 37°C with 39 μM nitroglycerin for 1 min and 5.5 mM *N*-acetylcysteine was added at time zero. The incubation mixture was sampled at frequent times after addition of *N*-acetylcysteine to determine the maximal rate of platelet aggregation in response to 11 μM ADP, a value that typically was derived from no more than the first 15 s of the aggregation tracing after addition of ADP. (Please note: the extent of aggregation was not measured in these experiments because the time required to achieve the maximal extent of aggregation was prohibitively lengthy [up to 2 min] relative to the time course of changes in cGMP that were being simultaneously measured.)

The concentration of nitroglycerin used (39 μM) was chosen to permit measurement of aggregation rates that were less than completely inhibited, thereby permitting assessment of changes in rates at early times. Since the earliest time point sampled after addition of nitroglycerin was 1 min, the large and transient increase in cGMP described by Mellion et al. (27) at very early times (<30 s) after addition of nitroprusside or nitric oxide would not have been detected if it occurred in platelets exposed to nitroglycerin. Only the stable, late-appearing

*Table II. Inhibition of Platelet Aggregation by Guanylate Cyclase Activators and Potentiation of Inhibition by N-Acetylcysteine**

Activator	IC_{50}	
	−*N*-Acetylcysteine	+*N*-Acetylcysteine
	μM	*μM*
Sodium azide	1.3	0.026
Nitroglycerin	42	0.80
Sodium nitroprusside	10	0.46
Sodium nitrite	59,000	680

* Aggregation of platelets in PRP was induced by addition of 11 μM ADP after incubating platelets at 37°C with various concentrations of azide, nitroglycerin, nitroprusside, or nitrite±5.5 mM *N*-acetylcysteine for 5 min. For further details, see Methods.

*Table III. Increase in Platelet cGMP by Nitroglycerin and Potentiation by N-Acetylcysteine**

[Nitroglycerin]	cGMP	
	−*N*-Acetylcysteine	+*N*-Acetylcysteine
	pmol/10^8 platelets	
0.0	0.20±0.04	0.22±0.04
3.9×10^{-10}	0.26±0.02	0.36±0.04
3.9×10^{-9}	0.32±0.04	0.53±0.02
3.9×10^{-5}	0.54±0.08	1.00±0.10
3.9×10^{-4}	0.80±0.04	4.08±0.26

* Platelets in PRP were incubated for 5 min at 37°C with a range of nitroglycerin concentrations±5.5 mM *N*-acetylcysteine. Each point represents the mean±SEM of three experiments done in duplicate. For further details, see Methods.

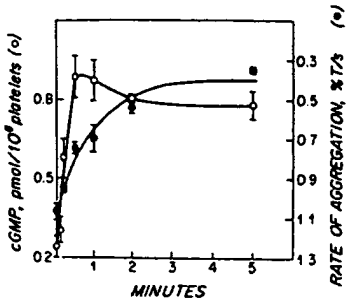

Figure 2. Time course of increase in platelet cGMP after addition of nitroglycerin and *N*-acetylcysteine. Platelets in PRP were incubated at 37°C with 39 μM nitroglycerin, and, at time zero, *N*-acetylcysteine was added to 5.5 mM. The incubation mixture was sampled at various times after addition of *N*-acetylcysteine, and then cGMP content (○) and maximal rate of aggregation (●) (maximal change in percent transmittance per second) determined. Each point represents the mean±SEM of two or three experiments done in duplicate. Note that the ordinate is inverted for the rate of aggregation.

(i.e., beyond 1 min) elevations of cGMP were measured in this experiment in order that they might be correlated better with changes in aggregation rate.

Fig. 2 shows that by 30 s after addition of 5.5 mM *N*-acetylcysteine to platelets incubated with 39 μM nitroglycerin, cGMP levels increased fourfold to 0.90 pmol/10^8 platelets. Maximal inhibition of platelet aggregation rate (plotted with a reversed ordinate for purposes of comparison with the cGMP plot) lagged behind and was not attained until 2.5 min after addition of *N*-acetylcysteine. Thus, this experiment demonstrates that the increase in platelet cGMP induced by nitroglycerin in the presence of *N*-acetylcysteine preceded the attainment of maximal inhibition of platelet aggregation.

Effect of nitroglycerin on reduced glutathione concentration in platelets. Nitroglycerin undergoes denitrification in smooth muscle cells and hepatocytes through the action of an organic nitrate reductase (28). This denitrification requires GSH, the thiol undergoing oxidation in the process. GSH levels were measured in the quiescent platelet and found to be 4.0×10^{-17} mol GSH/platelet, a value comparing favorably with published values (29). Incubating platelets with increasing concentrations of nitroglycerin led to a reduction in this intracellular GSH to <10% of the base-line levels at ~1 μM nitroglycerin (Fig. 3).

Effect of S-nitroso-N-acetylcysteine on platelet aggregation. *S*-nitroso-*N*-acetylcysteine, synthesized from *N*-acetylcysteine and $NaNO_2$ as described in Methods, markedly inhibited platelet aggregation in response to 11 μM ADP, as depicted in Fig. 4. Incubating PRP for 5 min with *S*-nitroso-*N*-acetylcysteine inhibited aggregation with an IC_{50} of 6 nM; it is important to note that at equivalent concentrations of *N*-acetylcysteine or $NaNO_2$ alone, no significant inhibition was noted (Fig. 1 and Table II), which supports the hypothesis that the adduct itself (and/or a metabolic product thereof) was the active species. Similar effects of *S*-nitroso-*N*-acetylcysteine were noted when using epinephrine (12.5 μM) and calf skin collagen (0.12 mg/ml) as agonists (Table IV).

Formation of S-nitroso-thiols by platelets on incubation with nitroglycerin. Platelets in PRP were incubated with 2.2 mM nitroglycerin with or without 5.5 mM *N*-acetylcysteine for 5 min at 37°C and extracted with TCA. The concentration of total nonprotein *S*-nitroso-thiol was determined by assaying free nitrite using diazotization and aromatic derivatization in the presence and absence of $HgCl_2$ as described in Methods. Mercuric ions catalyze the hydrolysis of the nitroso moiety from the nitroso-thiol adducts and, in so doing, permit the determination of nitroso-thiols. In the absence of *N*-acetylcysteine, 1.5±0.5 nmol/10^8 platelets of *S*-nitroso-thiol was detected, while with *N*-acetylcysteine, this value increased to 5.1±1.0 nmol/10^8 platelets. No nitroso-thiol was detected in the absence of nitroglycerin. The effect of lesser concentrations of nitroglycerin was not assessed because sensitivity of the assay was limited and prohibitively large amounts of platelets would have been required. Protein-associated *S*-nitroso-thiols were not measured in these experiments because only adducts to which the plasma membrane is permeable appear to be important for the effect of other organic nitrates noted in previous studies (27).

Discussion

These experiments demonstrate that the reduced thiol, *N*-acetylcysteine, markedly potentiates inhibition of platelet aggregation by nitroglycerin and other organic nitrate vasodilators.

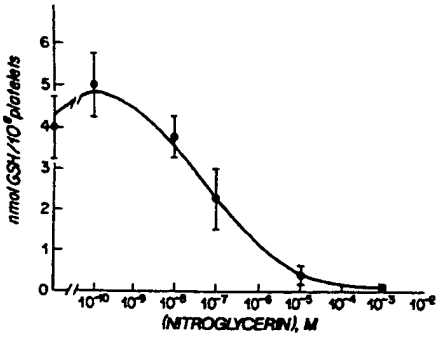

Figure 3. Effect of nitroglycerin on platelet GSH content. Platelets in PRP were incubated with increasing concentrations of nitroglycerin at 37°C for 1 min and the platelet GSH content determined as described in Methods. Longer incubations failed to reduce GSH values further. Each point represents the mean±SEM of three experiments done in duplicate.

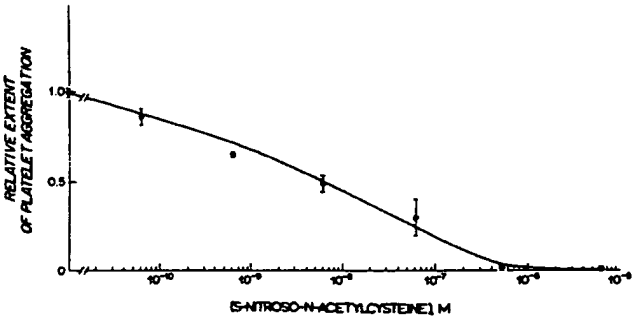

Figure 4. Effect of *S*-nitroso-*N*-acetylcysteine on ADP-induced platelet aggregation. Platelets in PRP were incubated for 5 min with increasing concentrations of *S*-nitroso-*N*-acetylcysteine at 37°C, after which aggregation was induced by addition of 11 μM ADP. Values on the ordinate reflect extent of aggregation relative to that in the absence of *S*-nitroso-*N*-acetylcysteine (corresponding to 85% maximal light transmittance). Each point represents the mean±SEM of three experiments done in duplicate.

*Table IV. Inhibition of Platelet Aggregation by S-Nitroso-N-Acetylcysteine**

Agonist	IC_{50}
	μM
ADP (11 μM)	0.0061±0.0005
Epinephrine (12.5 μM)	0.100±0.001
Collagen (0.12 mg/ml)	0.036±0.001

* Aggregation of platelets in PRP was induced by addition of agonist after incubating platelets at 37°C with various concentrations of *S*-nitroso-*N*-acetylcysteine for 5 min. Each point represents the mean±SEM of two experiments done in duplicate. For further details, see Methods.

N-Acetylcysteine was chosen for these experiments in place of other thiols because of its proved safety in humans and because of its demonstrated efficacy in potentiating the hypotensive effect of nitroglycerin in vivo (16). In smooth muscle cells, the relaxing effect of nitroglycerin was felt initially to be mediated by a nitroglycerin "receptor" (30), through which specific tolerance could be induced by prolonged exposure to nitroglycerin and reversed by reduced thiols (15). Further studies by Ignarro's group (31) suggested that the effect of nitroglycerin on smooth muscle cells was more complex than this and that cGMP was an essential mediator of nitroglycerin-induced vasodilatation. In these experiments, increases in smooth muscle cGMP in response to incubation with nitroglycerin preceded decreases in vascular tone, supporting the importance of the cyclic nucleotide.

In platelets, the inhibition of aggregation by nitroglycerin and other guanylate cyclase activators (nitroprusside, azide, and nitrite) suggests that cGMP is a crucial mediator of this inhibition (27). The fact that the maximal increase in platelet cGMP preceded the maximal inhibition of aggregation further supports this hypothesis. Controversy exists in the platelet literature about the role of cGMP in the aggregation response. Chiang and colleagues (32, 33), White et al. (34), and Glass et al. (35) demonstrated that platelet aggregation is associated with a rise in platelet cGMP levels, while the more recent studies of Claesson and Malmsten (36) and Weiss et al. (37) showed that cGMP either had no effect on platelet aggregation or else, at higher concentrations, inhibited aggregation. The data presented here show that, at least as far as inhibition of platelet aggregation by nitroglycerin is concerned, platelet cGMP levels maximally increase before maximal inhibition of aggregation, thereby arguing that elevation of cGMP is associated with inhibition of aggregation.

The ability of the reduced thiol, *N*-acetylcysteine, to potentiate the inhibitory effect of nitroglycerin further supports the importance of guanylate cyclase activity in the inhibition of platelet aggregation in these experiments. Preincubation with reduced thiol was essential for the expression of partially purified hepatic guanylate cyclase activity (38). In addition, the fact that hepatic and pulmonary (38) guanylate cyclase are rapidly inactivated by molecular oxygen, sulfhydryl oxidants, and thiol alkylating agents suggests that the redox state of sulfhydryl groups on guanylate cyclase determines its activation. Reduced thiols have also been found to stimulate activation of purified guanylate cyclase by nitroglycerin, among which are included cysteine, GSH, penicillamine, and dithiothreitol (26).

In the metabolism of nitroglycerin, denitrification occurs in a process requiring that GSH be catalyzed in liver by an organic nitrate reductase (30); the specific enzyme that serves this function is probably a glutathione-*S*-transferase (39). Denitrification is probably responsible not only for the metabolic fate of nitroglycerin, but also for its role as an activator of guanylate cyclase. Nitrite ion hydrolyzed from nitroglycerin reacts with reduced thiol to form *S*-nitroso-thiol compounds that, themselves, are very potent activators of guanylate cyclase (26), vasodilators (24), and, as shown both in this study for *S*-nitroso-*N*-acetylcysteine and in a recent study for other *S*-nitroso-thiols (40), extremely potent inhibitors of platelet activation. The loss of GSH and the appearance of *S*-nitroso-thiols in platelets incubated with nitroglycerin supports the existence of this mechanism in platelets. The chemical identity of the *S*-nitroso-thiol(s) that form intracellularly has yet to be determined, but the possible candidates include *S*-nitroso-glutathione, *S*-nitroso-cysteine, or, perhaps, *S*-nitroso-*N*-acetylcysteine itself.

What *N*-acetylcysteine does in this system is, as yet, not fully explained: it may serve as a source of thiol-reducing equivalents for the GSH system in the platelet; it may directly activate guanylate cyclase; it may serve as a reduced thiol source in the enzyme-catalyzed denitrification reaction; or it may serve as a substrate directly in the formation of *S*-nitroso-thiols. Further studies are currently underway to clarify these issues.

In summary, the data presented here show the following: that (*a*) nitroglycerin and other organic nitrates inhibit platelet aggregation and that this inhibition is potentiated markedly by the reduced thiol, *N*-acetylcysteine; that (*b*) nitroglycerin, as other guanylate cyclase activators, inhibits platelet aggregation by inducing an increase in platelet cGMP levels that rise maximally before maximal inhibition of platelet aggregation; that (*c*) *S*-nitroso-thiols form when platelets are incubated with nitroglycerin; that (*d*) *S*-nitroso-*N*-acetylcysteine is an extremely potent inhibitor of platelet aggregation; and that (*e*) such *S*-nitroso-thiols adducts are the active forms of nitroglycerin and other organic nitrates.

These data provide new insights into the possible effects of nitroglycerin on the vascular bed. At concentrations that are pharmacologically attainable in vivo, nitroglycerin reacts with GSH and/or other cellular thiols to form *S*-nitroso-thiols that not only vasodilate directly, but also markedly inhibit platelet aggregation. Inhibition of platelet activation and aggregation prevents synthesis of platelet-derived thromboxane A_2, and thereby further potentiates local direct vasodilating effects. In light of these data, the use of *S*-nitroso-*N*-acetylcysteine or other *S*-nitroso-thiols as antihypertensive or antiplatelet agents deserves further study.

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DRUG COMBINATIONS OBTAINED FROM ALPHA-LIPOIC ACID AND CARDIOVASCULAR-ACTIVE SUBSTANCES [ARZNEIMITTELKOMBINATIONEN AUS ALPHA-LIPONSÄURE UND HERZ-KREISLAUFAKTIVEN SUBSTANZEN]

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TITLE (54): DRUG COMBINATIONS OBTAINED FROM ALPHA-LIPOIC ACID AND CARDIOVASCULAR-ACTIVE SUBSTANCES

FOREIGN TITLE [54A]: ARZNEIMITTELKOMBINATIONEN AUS ALPHA-LIPONSÄURE UND HERZ-KREISLAUFAKTIVEN SUBSTANZEN

Description

The invention relates to pharmaceutical combination preparations consisting of alpha-lipoic acid, dihydrolipoic acid and their oxidized or reduced R- or S-enantiomers as well as metabolites of alpha-lipoic acid and at least an organic nitrate, calcium- antagonists or ACE-inhibitors.

Alpha-lipoic acid, chemically seen, is 1,2- dithia-cyclopentane-3- valerianic acid.

It is widely used in plants and animals in the form of R-enantiomer and acts as a co-enzyme in many enzymatic reactions, represents a growth factor for some bacteria and protozoa and is used for amanita phalloides poisoning. Further, alpha-lipoic acid- racemate has antiphlogistic, antinociceptive (analgesic) as well as cyto-protective, neuro-protective, antiallergic and anti-tumor properties (refer to DE 40 35 442 A1).

The purely optical enantiomers of alpha-lipoic acid (R- and S-form, that means R-alpha- lipoic acid and S-alpha- lipoic acid) have special effects when compared to the racemate.

It is known that R-enantiomer is effective predominantly as antiphlogistic and the S-enantiomer as predominantly antinociceptive. The antiphlogistic effect of

R-enantiomer is about 10 times stronger than that of racemate.

The antinociceptive (analgesic) effect of S-enantiomer is for example, 6 times stronger than that of racemate. Therefore, the enantiomers represent active substances that are much more specific and stronger when compared to the racemate (refer to DE 40 35 442 A1).

/2 Numbers in the margin indicate the pagination in the foreign text.

The therapeutically important organic nitrates are esters of nitrous acid and nitric acid such as amylnitrite, nitroglycerine, isosorbitdinitrate or 5-isosorbitmononitrate. The basic effect of nitrites and organic nitrates is the relaxation of smooth musculature (effective mechanism of organic nitrate refer to Forth, Henschler, Rummel in: Pharmacology and toxicology, Scientific journal, Mannheim, Wien, Zurich, 5th edition 1990, Pages 268 onwards).

It is known that

- the effect of nitrites and organic nitrates on smooth musculature is linked to the availability of SH-groups
- the S-nitrosothiols bring about relaxation of coronary vascular streaks

- the instability of S-nitrosothiols correspond to the duration of effect of organic nitrates in their chronological sequence in case of i.v. administration and
- Slowdown of effectiveness was observed in spite of constant dosage during continuous therapy leading to so-called nitrate tolerance, above all, during retard- preparations in higher dosage and for transdermal administration with persistently high organic nitrate blood levels.

Calcium - antagonists as combination partners are Verapamil, nifedipine, nimodipine, felodipine, isradipine, nitrendipine, nisoldipine, nicardipine, nivaldipine and diltiazem. Use of calcium antagonists, nimodipine for fighting against impairments of peripheral nerves that were caused due to diabetes, were already described in DE 41 25 116 A1.

ACE- inhibitors of the type of captopril, lisinopril, perindropril-tert-butylamine, ramipril and enalapril hydrogen maleate can be used as other combination partners.

ACE- inhibitors are angiotensin-converting- enzyme-inhibitors; they are compounds whose effect is based on the hindrance of splitting of a peptide bond of angiotensin I in the vascoconstricting angiotensin II.

Thus, a decrease in systemic resistance and increase in cardiac output and decrease in left-ventricular and right-ventricular filling pressure are caused.

Oxyfedrin can be used as another combination partner. Oxyfedrin is an aminoketone obtained from the phenylethylamine- series (oxyfedrine = L-3- (β-hydroxy-alpha-methylphenethylamino)- 3'-methoxy-propiophenone-hydrochloride). The cardio-energetic coronary effect of Oxyfedrine lies in improvement of coronary- and myocardial blood supply and increase in stroke volume and minute volume, whereby the cardiodynamic changes are correlated in each phase to optimum energy preparation and energy utilization.

The objective of the present invention is to make available combination preparations with synergetic effect for improved treatment of especially cardio-circulatory disorders as well as diseases caused due to diabetes.

It is achieved according to the invention in that pharmaceutical combination preparations, consisting of alpha-lipoic acid, dihydrolipoic acid and their oxidized or reduced R- or S-enantiomers as well as metabolites of alpha- lipoic acid, such as 6,8- bisnorlipoic acid, tetranorlipoic acid (active substance A) and at least an

organic nitrate, calcium- antagonists, ACE- inhibitors or oxyfedrine, are used.

For example, glyceroltri- nitrate, isosorbitdinitrate or 5-isosorbitmononitrate can be used as organic nitrates.

Verapamil, nifedipine, nimodipine, felodipine, isradipine, nitrendipine, nisoldipine, nicardipine, nivaldipine and diltiazem are suitable as calcium-antagonists.

Those of the type of captopril, lisinopril, perindropril-tert-butylamine, ramipril and enalapril hydrogen maleate can be used as ACE- inhibitors.

Surprisingly, it was found that stronger effects were obtained in the combination of say, glyceroltri- nitrate, isosorbitdinitrate or 5-isosorbitmononitrate with purely optical enantiomers of alpha- lipoic acid when compared to the racemate of alpha-lipoic acid alone.

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The combination of isosorbitdi-nitrate with S-enantiomer of alpha- lipoic acid shows vascular-relaxing effect and the R-enantiomer in combination with glyceroltri-nitrate shows anti-ischemic effect, whereby, surprisingly, the anti-ischemic effect of R-enantiomer in combination with 5-isosorbitmononitrate is stronger than

that of racemate of alpha- lipoic acid. The anti-ischemic effect of R-enantiomers in combination with isosorbitdi-nitrate is likewise stronger than that of racemate of alpha- lipoic acid. The enantiomers of alpha-lipoic acid in combination with organic nitrates such as glyceroltri-nitrate, isosorbitdinitrate or 5-isosorbitmononitrate, have active substances that are more specific and stronger when compared to the racemate of alpha- lipoic acid.

It was even more surprising that in the combination of active substances of Claim 3 such as the nitrates, say, glyceroltrinitrate, isosorbitdinitrate or 5-isosorbitmononitrate with the purely optical isomers of alpha- lipoic acid (R- and S-form, that means R-alpha-lipoic acid and S-alpha- lipoic acid) when compared to the organic nitrates alone, surprisingly, vascular-relaxing effect was found in the combination with say, the nitrate, isosorbitdintrate with R-enantiomer and the R-enantiomer is effective as anti-ischemic in combination with the nitrate, glyceroltrinitrate, whereby, surprisingly, the anti-ischemic effect of R-enantiomers in combination with nitrates, such as 5-isosorbitmononitrate, is stronger than that of the respective organic nitrate alone. The anti-ischemic effect of R-enantiomers in combination with nitrate such as isosorbitdintrate is say, stronger than

that of isosorbitdintrate. The enantiomers of alpha- lipoic acid in combination with nitrates such as glyceroltrinitrate, isosorbitdinitrate or 5-isosorbitmononitrate have much more specific and stronger active substances when compared to the organic nitrates of Claim 3.

The combinations with the organic nitrates according to the invention like, for e.g. glyceroltrinitrate, isosorbitdinitrate or 5-isosorbitmononitrate and the optical enantiomers of alpha-lipoic acid exhibit a good coronary- relaxing, anti-ischemic, cardiac insufficiency-therapeutic or anti-organic nitrate tolerance- effect in the following trial models:

1) in vitro: isolated guinea pig- rabbit aorta or isolated right or left auricle of guinea pig
2) in vivo: dog, domestic pig, model: coronary stenosis with the help of balloon-tipped catheter methods with subsequent, histological trial for reducing size of infarction or occurrence of infarction.

The pharmaceutical combination preparations consisting of active substances A and an organic nitrate, generally contain between 1 mg to 1.2 g as single dose, preferably 2 mg to 800 mg of R- or S-alpha- lipoic acid in combination with preferably 0.1 -40 mg of organic nitrate, such as for

e.g. glyceroltrinitrate, isosorbitdinitrate or 5-isosorbitmononitrate.

The obtained effective ranges per kg of body weight must lie between 1.5 and 200 mg, preferably between 4 and 100 mg for R- or S-alpha- lipoic acid in the combination according to the invention, and must lie between 0.1 - 40 mg, preferably 0.8 - 20 mg for the organic nitrates, like, say glyceroltrinitrate, isosorbitdinitrate or 5-isosorbitmononitrate.

Likewise, it was surprisingly found that stronger effects were obtained in the combination of calcium-antagonists, for e.g. Verapamil, with purely optical enantiomers of alpha-lipoic acid when compared to the racemate of alpha-lipoic acid alone. The combination with say, calcium- antagonists, verapamil with R- enantiomers shows anti-diabetic effect, i.e. blood sugar-decreasing effect and the R-enantiomer in combination with calcium-antagonists, nimodipin, shows neurocytoprotective effect. The neuroprotective effect of R- enantiomer in combination with calcium- antagonists, nimodipin, is stronger than that of the racemate of alpha- lipoic acid. The anti-hypertensive effect of R- enantiomer in combination with calcium- antagonists, nimodipin or nifedipin is, likewise, stronger than that of the racemate of alpha- lipoic acid.

The enantiomers of alpha- lipoic acid in combination with nifedipin constitute very much specific and stronger effective active substances when compared with the racemate of alpha- lipoic acid.

It was surprisingly found that in the combination of active substances of Claim 4 like, for e.g., calcium antagonists, Verapamil with purely optical R-isomers of alpha-lipoic acid when compared to Verapamil alone, surprisingly, it was more anti-diabetic, i.e. it had blood sugar- reducing effect in the combination and the R-enantiomer in combination with calcium-antagonists, nimodipin, shows stronger neurocytoprotective effect than nimodipin alone.

The anti-hypertensive effect of R-enantiomer in combination with calcium antagonists, nifedipin, is stronger than that of nifedipin alone. The enantiomers in combination with the calcium- antagonists of the type verapamil, nifedipine, nimodipine, felodipine, isradipine, nitrendipine, nisoldipine, nicardipine, nivaldipine and diltiazem constitute much more specific and stronger effective substances when compared to the calcium antagonists of Claim 4 alone.

The pharmaceutical preparations of combinations of active substances A with at least one calcium- antagonist generally contain between 1 mg to 3 g as single dose, preferably 2 mg to 1.2 g of R- or S-alpha- lipoic acid in combination with 1 to 120 mg of a calcium- antagonist.

The obtained effective ranges per 1 kg of body weight must lie between 1.5 and 200 mg, preferably between 4 and 100 mg for R- or S-alpha- lipoic acid in the combination according to the invention, and must lie between 1 - 120, preferably between 5- 90 mg for the calcium- antagonist.

Further, it was surprisingly found that stronger blood sugar-reducing effects occur in the combination with ACE-inhibitors, like Captopril, with the purely optical R-enantiomer of alpha- lipoic acid when compared to the racemate of alpha- lipoic acid and stronger cardio-cytoprotective effects occur with the R-enantiomer in combination with ACE-inhibitor, enalapril hydrogen maleate. The cardio-cytoprotective effect of enantiomer in combination with ACE-inhibitor, enalapril hydrogen maleate, is stronger than that of the racemate of alpha- lipoic acid.

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The anti-hypertensive effect of R- enantiomer in combination with the ACE-inhibitor, ramipril, is likewise, stronger than that of the racemate of alpha- lipoic acid.

The enantiomers of alpha- lipoic acid in combination with the ACE-inhibitors of the type captopril, lisinopril, perindropril-tert-butylamine, ramipril, enalapril hydrogen maleate constitute much more specific and stronger effective substances when compared to the racemate of alpha- lipoic acid.

It was surprisingly found that vascular-relaxing effect was obtained with the R-enantiomer surprisingly in combination with, say, the ACE-inhibitor, Captopril in combination of active substances of Claim 5 as with the ACE-inhibitors, such as captopril, ramipril and enalapril hydrogen maleate with the purely optical isomers of alpha-lipoic acid (R- and S-form, that means R-alpha- lipoic acid and S-alpha- lipoic acid) when compared to the organic ACE-inhibitors alone and the R-enantiomer in combination with the nitrate, glyceroltrinitrate is cardiocytoprotective, whereby, surprisingly, the cardiocytoprotective effect of R- enantiomer in combination with ACE-inhibitor such as for e.g. captopril, is stronger than that of captopril alone. The cardiocytoprotective effect of R-enantiomer in combination with ACE-inhibitor such as say captopril, is

stronger than that of captopril alone. The enantiomers of alpha-lipoic acid in combination with ACE-inhibitors such as captopril, ramipril, lisinopril and enalapril hydrogen maleate, therefore, constitute much more specific and stronger effective substances when compared to the ACE-inhibitors of Claim 5.

The effects of combinations of the invention with ACE-inhibitors were investigated with the following trial models:

1) in vitro: isolated guinea pig- rabbit aorta or isolated right or left auricle of guinea pig
2) in vivo: dog, domestic pig, model: coronary stenosis with the help of balloon-tipped catheter methods with subsequent, histological trial for reducing size of infarction or occurrence of infarction.
3) Test model for cardio-cytoprotective effect

For instance, the S- enantiomer (S- alpha-lipoic acid) in combination with ACE- inhibitor, captopril, shows a synergistic, prophylactic and therapeutic effect of

combination pointing to the vascular-relaxing effect on humans and isolated guinea pig aorta which can be thought of for those of alpha- lipoic acid alone or the ACE-inhibitor alone (peroral application).

4) Testing of anti-arteriosclertoic effect

Scalbert et al: Journal of Cardiovascular Pharmacology 18 (Suppl. 7) Page 25 - Page 32 1991

5) Test model for anti-hypertensive effect

(Model refer also: Nagano et al.: Journal of Hypertension 1991, 9; 595- 599

For instance, the S-enantiomer in combination with ACE- inhibitor, lisinopril, shows a synergistic, prophylactic and therapeutic effect of combination pointing to hypertension in spontaneously- hypertensive rats, which can be thought of for those of alpha-lipoic acid (that means the racemate) or the ACE-inhibitor alone (peroral application). Likewise, an anti-hypertensive effect was observed in animal trials for the oxidized or reduced R- and S-form of alpha- lipoic acid in combination with ACE-inhibitor, enalapril, already from a dose of 20 mg/kg of R- or S-enantiomer or alpha- lipoic acid in combination with 10 mg/kg of ACE-inhibitor by mouth.

6) Test model for anti-diabetic effect

Streptozotocin- induced diabetes in rats. Study of glucose assimilation in muscles after pre-treatment of rats with the combination.

7) Testing for acute cell toxicity in fibroblasts of mouse, L 929 or the like. According to LINDL et al.

in: Cell and tissue culture, Gustav Fischer Verlag, Stuttgart, New York, 2nd Edition, 1989, Pages 164- 167.

8) Testing for influence of substance on metabolic activity

According to LINDL et al. in: Cell and tissue culture, Gustav Fischer Verlag, Stuttgart, New York, 2nd Edition, 1989, Pages 167- 168.

The combination of active substances A with ACE-inhibitors shows a good effect on metabolic activity in the following trial models:

- rats -or guinea pig aorta- model
- in-vitro model: Isolated aorta of rats or guinea pigs with prophylactic administration of test substances

For methods, refer also: Tian et al: European Journal of Pharmacology, 203, (1991) 71- 77

9) Testing for anti-arteriosclerotic effect

Scalbert et al.: Journal of Cardiovascular Pharmacology 18 (Suppl. 7) Page 25- 32, 1991.

The pharmaceutical combination preparations consisting of the active substance A and at least one ACE-inhibitor generally contain between 1 mg to 3 g as

single dose, preferably 2 mg to 1.2 g of R- or S-alpha-lipoic acid and for instance, 1 to 18 mg of captopril.

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The active substances must be released from the preparations slowly.

Preferred forms of usage are for e.g., tablets that contain between 5 mg and 500 mg or solutions that contain between 5 mg to 0.2 g/ml of fluid of active substances.

Further, it was found surprisingly that vascular-relaxing effect was found with R-enantiomer in combination with oxyfedrin in the combination of oxyfedrin with purely optical isomers of alpha-lipoic acid (R- and S-form, that means R-alpha-lipoic acid and S-alpha-lipoic acid) when compared to the racemate of alpha-lipoic acid alone and the R-enantiomer in combination with oxyfedrin is effective as an anti-ischemic, whereby, surprisingly, the anti-ischemic effect of R-enantiomer in combination with oxyfedrin is likewise stronger than that of the racemate of alpha-lipoic acid. The anti-ischemic effect of R-enantiomer in combination with oxyfedrin is likewise stronger than that of racemate of alpha- lipoic acid.

The enantiomers of alpha-lipoic acid in combination with oxyfedrin, therefore, constitute much more specific

and stronger active substances when compared to the racemate of alpha- lipoic acid.

Preferably, the salts with pharamaceutically usable halogens are used in aqueous solutions.

The application of combination preparations consisting of active substance A and at least one organic nitrate, calcium- antagonists, ACE-inhibitors or oxyfedrin can be undertaken on the skin or mucous membrane or inside the body, for example, orally, enteral, pulmonal, nasal, lingual, intravenous, intra-arterial, intra-cardial, intra-muscular, intra-peritoneal, intracutaneous, subcutaneous. It concerns sterile or sterilized products in case of parenteral administration.

Therefore, the administration can be undertaken in the form of tablets, capsules, pills, sugar-coated pills, aerosols, salves, creams, medical strips or in fluid form, whereby the active substances can be combined, if necessary, with known excipients.

The fluid forms of administration can be: alcoholic or aqueous solutions as well as suspensions and emulsions.

Salts with pharmaceutically usage halogens are preferably used in aqueous solutions.

The customary bases or cations can be used as halogens which are physiologically compatible in the salt form. Examples for the same are alkali metals or alkaline- earth metals, ammonium hydroxide, alkaline amino acids such as arginine and lysine, amines of formula N R_1 R_2 R_3 wherein the radicals R_1 R_2 and R_3 are same or different and hydrogen, C_1 - C_4- alkyl or C_1 - C_4- oxyalkyl mean mono and diethanolamine, 1-amino-2-propanol, 3- amino-1- propanol; alkylene diamine with an alkylene chain made from 2 to 6 C-atoms such as ethylene diamine or hexamethylene tetramine, saturated cyclic amino compounds with 4 - 6 ring carbon atoms such as piperidine, piperazine, pyrrolidine, morpholine, N-methyl glucamine, creatine, trometamol.

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Table 1

Examples for oral doses of combination preparations with organic nitrates for therapy of angina pectoris in humans

Organic nitrate	Active substance A	Daily dosage of organic nitrate	Daily dosage of active substance A	Single dosage a) organic nitrate b) Active substance A	Frequency of application
Glycerol-	Oxidized/	0.8-	300-1200	a) 0.8 -	1-3

trinitrate	reduced racemate or R- or S-alpha-lipoic acid	6.5 mg/d	mg	6.5 mg b) 100-400 mg	
Isosorbit-dinitrate	Oxidized/ reduced racemate or R- or S-alpha-lipoic acid	10- 30 mg/d	300-1200 mg	a) 2.5 - 10 mg b) 100-400 mg	1- 3
Isosorbit mono-nitrate	Oxidized/ reduced racemate or R- or S-alpha-lipoic acid	20- 80 mg/d	300-1200 mg	a) 10 - 40 mg b) 100-400 mg	1- 2

The single dose of active substance A in the combination with organic nitrates can contain the following for R-enantiomer of alpha- lipoic acid:

a) for oral administration between 5 - 300 mg, preferably 30 mg -240 mg, especially 30 mg - 150 mg

b) for parenteral administration (for e.g. intravenous, intramuscular) between 10 - 250 mg, preferably 20 mg - 150 mg, especially 30- 90mg.

The doses can be administered daily once to 4 times, preferably 1- 2 times. The daily dosage of R- or S-alpha-lipoic acid in combination with organic nitrates can be 2-

40mg/kg weight for humans, whereby this dose is administered up to 4 times per day.

The daily dose can be for instance, 100 - 600 mg. Therefore, the drugs preferably contain 100 - 600 mg of R- or S- alpha-lipoic acid in a galenic formulation, whereby such a dose is preferably administered up to 3 times.

The single dose of organic nitrates in the combination preparations can lie for instance:

a) for oral administration between 5mg - 40 mg, preferably 10 mg.

b) for inhalation (solutions of aerosols) between 0.100 mg - 1.2 mg, preferably 0.41 mg - 1 mg.

In case the organic nitrate such as glyceroltri-nitrate, isosorbitdi-nitrate or 5-isosorbitmono-nitrate is used for instance, in combination with R- or S-alpha-lipoic acid in the form of its salts, the halogen can be used even in excess, that means, in a higher quantity than equimolar quantity.

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Table 2

Example for oral doses of combination preparations with calcium-antagonists for the therapy of different indications in humans

Substance of Claim 2	Substance of Claim 1	Daily dose of substance of claim 2	Daily dose of substance of claim 1	Single dose of substance of a) claim 2 b) claim 1	Frequency of application	Indication
Nifedipin	Oxidized/ reduced racemate or R- or S- enantiomer of alpha-lipoic acid	5-30 mg/day	300 mg- 1.2 g	a) 5-10 mg b) 100 mg - 400 mg	1-3	Angina pectoris, hypertension
Verapamil	Oxidized/ reduced racemate or R- or S- enantiomer of alpha-lipoic acid	40- 360 mg/day	300 mg- 1.2 g	a) 40 mg - 120 mg b) 100 mg- 400 mg	1- 3	Tachycardia, atrial fibrillation, ischemic-ventricular extrasystole after myocardial infarction, coronary-insufficiency
Nimodipin	Oxidized/ reduced racemate or R- or S- enantiomer of alpha-lipoic acid	30- 90 mg/day	300 mg- 1.2 g	a) 10 mg - 30 mg b) 100 mg - 400 mg	1- 3	Neuropathy, cerebral neuropathy
Diltiazem	Oxidized/ reduced racemate or R- or S- enantiomer of alpha-lipoic acid	60- 180 mg/day	300 mg- 1.2 g	20- 60 mg b) 100 mg- 400 mg	1-3	Coronary heart disease, coronary spasm, atrial fibrillation
Felodipin	Oxidized/	5-10	300 mg-	a) 5 mg-	1	Angina

	reduced racemate or R- or S- enantiomer of alpha- lipoic acid	mg/day	1.2 g	10 mg b) 100 mg- 400 mg		pectoris, myocardial infarction, hypertension, coronary heart disease
Nisoldipin	Racemate or R- or S- enantiomer of alpha- lipoic acid	5- 10mg/day	250 mg- 1.2 g	a) 10 mg - 20mg b) 60 mg - 400 mg	1-2	Coronary heart disease, hypertension, angina pectoris

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The single dose of active substance A in combination, for instance, with calcium- antagonist, nimodipin, can lie:

a) for oral administration between 50 mg - 3g, preferably 100 mg - 1.2 g, especially 300- 600 mg.

b) for parenteral administration (for e.g. intravenous, intramuscular) between 50 mg - 2 g, preferably 100 mg - 3 g, especially 300 - 600 mg.

c) for inhalation (solutions or aerosols) between 0.010 mg - 1.2 g, preferably 0.020 mg- 600 mg, especially 0.5 - 300 mg.

The doses can be administered one to four times, preferably one to three times daily or even as continuous infusion with the help of infusionates.

The daily dose of R- or S-alpha- lipoic acid in combination with nimodipin can lie at 2- 40 mg per kg of

weight in humans, the single dose being 1- 10 mg per kg of weight.

The daily dose can be preferably 100 - 600 mg, therefore, the drugs contain 100- 600 mg of R- or S- alpha-lipoic acid in a galenic formulation, whereby such a dose is administered preferably up to 4 times.

For the treatment, say 1 to 3 tablets with a content of 5 mg to 2 g of the active substance A can be given daily or say, an ampoule/ infusion bottle of 1 to 100 ml of content with 500 mg to 6 g of active substance A in combination with 1 mg - 20 mg of a calcium-antagonist is recommended daily one to four times in case of intravenous injection.

In case of oral administration, the minimum daily dose of active substance A in combination with a calcium-antagonist is, say, 200 mg; the maximum daily dose should not exceed 1 g.

The single dose of a calcium-antagonist, such as nimodipin, in combination with R- or S- enantiomer of alpha-lipoic acid, can lie at:

a) for oral administration between 30 - 90 mg, preferably 10 mg - 80 mg, especially 30- 60 mg.
b) for parenteral administration (for e.g. intravenous) about 15 micrograms/ kg of body weight/ hour every hour.

The daily oral dose of calcium-antagonist, nimodipin, in combination with the oxidized or reduced racemate or R- or S-enantiomer of alpha- lipoic acid can lie at 0.05- 1.2 mg/ per kg of body weight in humans, the single dose of nimodipin in this combination is at 0.005 - 0.04 mg per kg of body weight, whereby this dose is administered suitably up to three times a day.

For the treatment, 1 to 2 tablets with a content of 1 mg to 30 mg of nimodipin can be given daily three times for the calcium- antagonist, nimodipin in the combination of the invention or say, an ampoule/ infusion bottle of 10 to 50 ml of content with 0.10 mg to 10 mg of nimodipin is recommended daily one to three times in case of intravenous injection.

In case of oral administration, the minimum daily dose for nimodipin in combination is 30 mg; however, the maximum daily dose should not exceed 360 mg.

Preferred forms of administration of combination partner A with nimodipin are for e.g. tablets that lie between 10 mg and 2 g or solutions that contain between 10 mg to 0.2 g/ml of fluid of active substances.

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Table 3

Example for oral doses of combination preparations with calcium-antagonists for the therapy of different indications in humans

ACE-inhibitor	Active substance A	Daily dose of ACE-inhibitor	Daily dose of active substance A	Single doses of a) ACE-inhibitor b) active substance A	Frequency of application	Indication
Captopril	Oxidized/ reduced racemate or R- or S- lipoic acid	1-50 mg/day	300 mg-1.2 g	a) 25- 50 b) 100 - 400 mg	1- 2	Cardiac insufficiency, Diabetes mellitus Type II, nephropathy, arteriosclerosis, hypertension
Ramipril	Oxidized/ reduced racemate or R- or S-alpha-lipoic acid	1- 10 mg/day	300 mg-1.2 g	a) 1 - 5 mg b) 100 - 400 mg	1- 2	Hypertension, heart weakness
Lisonopril	Oxidized/ reduced racemate or R- or S- alpha-lipoic acid	1- 20 mg/day	300 mg-1.2 g	a) 1 - 12 mg b) 100 - 400 mg	1- 3	Heart weakness, hypertension
Enalapril hydrogen maleate	Oxidized/ reduced racemate or R- or S- alpha-lipoic acid	1- 20 mg/day	300 mg-1.2 g	a) 2.5 - 20 mg b) 100 - 400 mg	1-2	Cardiac insufficiency, Diabetes mellitus Type II, nephropathy, arteriosclerosis, hypertension
Perindopril-tert-butylamine	Oxidized/ reduced racemate or R- or S- alpha-lipoic acid	1-8 mg/day	300 mg-1.2 g	a) 1- 4 mg b) 100-400 mg	1- 2	Cardiac insufficiency, Diabetes mellitus Type II, nephropathy, arteriosclerosis, hypertension

The single dose of active substance A in combination, for instance, with ACE-inhibitor, captopril, can lie:

a) for oral administration between 50 mg - 3g, preferably 100 mg - 1.2 g.

b) for parenteral administration (for e.g. intravenous, intramuscular) between 50 mg - 3 g, preferably 100 mg - 2 g.

For instance, the daily dose of R- or S-alpha- lipoic acid in combination with Captopril can lie at 2- 40 mg per kg of weight in humans, the single dose being 1- 10 mg per kg of weight.

The daily dose can be 100 - 600 mg, therefore, the combination preparations contain preferably 100- 600 mg of R- or S- alpha-lipoic acid in a galenic formulation, whereby such a dose is administered preferably up to 4 times.

For the treatment, say 1 to 3 tablets with a content of 2.5 mg to 2 g of the active substance A can be given daily or say, an ampoule/ infusion bottle of 1 to 100 ml of content with 500 mg to 6 g of active substance A in combination with 1 - 20 mg of an ACE-inhibitor, say, lisinopril, is recommended daily one to three times in case of intravenous injection.

In case of oral administration, the minimum daily dose of active substance A in combination with an ACE-inhibitor

is, say, 200 mg; the maximum daily dose should not exceed 1.2 g in case of oral administration.

The single dose of an ACE-inhibitor in combination with the active substance A, can lie between 1mg- 50 mg, preferably 2 mg- 25mg, for instance, for ACE-inhibitor, captopril in case of oral administration.

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For the treatment, 1 to 2 tablets with a content of 0.5 mg to 5 mg of an ACE-inhibitor, for e.g. ramipril is recommended daily one to two times in the combination. For oral administration, the minimum daily dosage is 1.5 mg, for instance,s for an ACE-inhibitor, ramipril in the combination; the maximum daily dose should not exceed 10 mg of ramipril.

The daily oral dose of ACE-inhibitor, ramipril, in combination with the oxidized or reduced racemate or R- or S-enantiomer of alpha- lipoic acid can lie at, say, 1.25-10 mg/ per day in humans, the single dose of ACE-inhibitor, ramipril, in the combination is at 0.5 - 5 mg per day, whereby this dose can be administered suitably up to two times per day.

The pharmaceutical preparations of the combination of active substance A with oxyfedrin generally contain between 1 mg to 1.2 g as single dose, preferably 2 mg to 800 mg of

R- and/or S-alpha-lipoic acid, for instance, in combination with preferably 4- 48 mg, especially 8 to 24 mg of oxyfedrin. The obtained effective range/ kg of body weight must lie between 1.5 and 200 mg, preferably between 4 and 100 mg, especially between 8 and 70 mg/kg for the R- or S-form of alpha- lipoic acid and for instance, for oxyfedrin, between 4- 48 mg, preferably 8-24 mg, especially between 8-16 mg. The active substances must be released slowly from the preparations.

Table 4

Example for oral doses for therapy of angina pectoris in humans

Substance of Claim 6	Substance of Claim 1	Daily dosage of substance of Claim 1	Daily dosage of substance of Claim 2	Single doses of substance of a) Claim 6 b) Claim 1	Frequency of application
Oxyfedrin	Oxidized/ reduced racemate or R- or S-enantiomer of alpha-	4- 48 mg/ d	300 mg- 1.2 g	a) 4 -24 mg b) 100- 400 mg	1-3

	lipoic acid				

The single dose of active substance of combination partner according to Claim 1 in combination with oxyfedrin can lie in the following range for R- enantiomer of alpha-lipoic acid:

a) for oral administration between 5 - 300 mg, especially 30 mg - 240 mg, especially 30 mg -150 mg.

b) for parenteral administration (for e.g. intravenous) between 10 -250 mg, preferably 20- 150 mg, especially 30 -90 mg.

The doses according to a) to c) can be administered, for instance, one to 4 times, preferably one to two times daily.

The single dose of active substance of combination partner according to Claim 6 in the combination, for instance, with oxyfedrin can lie:

a) for oral administration between 4 - 48 mg, preferably 8 - 24 mg.

b) for parenteral administration (for e.g. intravenous, intramuscular) between 4 -48 mg/day, preferably 0.3 mg/kg of body weight/ h mg/hour.

The daily dose of R- and/or S-alpha- lipoic acid in the combination can lie for instance with oxyfedrin at 4-48 mg for humans; the single dose for example, at 4- 24 mg, whereby this dose is administered suitably up to 3 times per day.

The daily dose can be preferably, for instance, 100 - 600 mg. Therefore, the drugs preferably contain 100-600 mg of R- and/or S-alpha-lipoic acid in a galenic formulation, whereby such a dose is preferably administered up to 3 times. For the treatment, 1 to 3 tablets with a content of 5 mg to 1.2g of active substance of Claim 1 can be administered three times daily or for instance, an ampoule/infusion bottle of 1 to 100 ml of content with 250 mg to 800 mg of active substance of Claim 1 in combination with 0.1 -10 mg/hour of active substance of Claim 2 is recommended daily once in case of intravenous injection.

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In case of a product for separate usage, it is also possible not to administer both the combination partners simultaneously. In such cases, for example, oxyfedrin can be administered intravenous and R- and/or S-alpha-lipoic acid can be administered as continuous infusion.

The general dosage range for the combination of the oxyfedrin mentioned above with R- and or S-alpha- lipoic acid

for the preventive effect in angina pectoris can be:

4- 24 mg /day of oral oxyfedrin, in combination with 1- 100 mg/kg of R- or S-enantiomer of alpha- lipoic acid;

For the coronary- therapeutic effect can be:

4- 48 mg/day of oral oxyfedrin in combination with 1- 100 mg/kg of R- or S-enantiomer of alpha- lipoic acid,

For the anti-anginal effect can be:

8- 48 mg/day of oral oxyfedrin in combination with 1 -100 mg/kg of R- or S-enantiomer of alpha-lipoic acid.

The combinations with oxyfedrin and the optical isomers of alpha-liponic acid show, for instance, a good coronary-relaxing, anti-ischemic, heart insufficiency-therapeutic effect for the following trial models:

For testing of preventive or therapeutic effect:

1) in vitro: isolated guinea pig- rabbit aorta or isolated right or left auricle of guinea pig.

For testing of preventive or therapeutic effect:

2) in vivo: dog, domestic pig, model: coronary stenosis with the help of balloon-tipped catheter methods with subsequent, histological trial for reducing size of infarction or occurrence of infarction.

The pharmaceutical preparations that contain oxyfedrin as active substance in combination with R-alpha- lipoic acid or S-alpha-lipoic acid, can be formulated say, in the form of aerosols, tablets, capsules, pills or sugar-coated pills, granulates, pellets, medical strips, solutions or emulsions, whereby the active substances can be combined, if necessary, with the relevant excipients.

For instance, the R-alpha-lipoic acid and the S-alpha-lipoic acid in combination with oxyfedrin can be applied especially in the form of a solution, for instance, peroral, topical, parenteral (intravenous, intra-articular, intramuscular, subcutaneous), inhalative, transdermal.

In case solutions are used, the optical isomers of alpha- lipoic acid and the oxyfedrin contained in the combination are used preferably in the form of a salt.

The doses specified above always refer to combinations with oxyfedrin with, for e.g., free optical isomers of alpha-lipoic acid. In case the optical isomers of alpha-lipoic acid is used in the form of a salt, the specified doses/ dose ranges are to be increased correspondingly because of higher molar-weight.

In the combination of the invention consisting of active substance A and at least one organic nitrate, calcium- antagonists, ACE-inhibitor or oxyfedrin, both

components can exist as a mixture. Generally, the components exist separately in a galenic formulation.

For instance, one component can exist as a tablet or coated tablet, while the other component as powder, both in a capsule and vice versa one component in the form of medical strips or aerosols or pellets, the other as powder, sugar-coated pills or tablets and vice versa and whereby, both the forms can exist, say, in a capsule; or in the form of multi-layered tablets or coated tablets.

The combination according to the invention can exist even as a product in which both the individual active substances exist in completely separated formulations, whereby, the active substance A or even both the active substances are contained in ampoules and/or infusion bottles, such that even a separate or a staggered administration is possible.

In case such completely separated formulations exist, these are to be coordinated with each other and must contain the respective active substances in the dosing unit in the same quantity and appropriate weight ratio in which they can exist in the combined mixture.

For a product with separate usage, it is also possible not to administer both the combination partners simultaneously. In such cases, an organic nitrate can be

administered intravenous and the R- and/or S-alpha- lipoic acid can be administered as continuous diffusion.

The general dosage range for the organic nitrates, such as glyceroltri-nitrate, isosorbitdi-nitrate or 5- isosorbit mono-nitrate with the active substance A, such as R- or S-alpha-lipoic acid can be

for the preventive effect in angina pectoris:

-0.8- 2.5 mg /day of oral glyceroltri-nitrate in combination with 1- 100 mg/kg of R- or S-enantiomer of alpha- lipoic acid;

for the coronary- therapeutic effect:

-2.5 - 40 mg/day of oral isosorbitdi-nitrate in combination with 1- 100 mg/kg of R- or S-enantiomer of alpha- lipoic acid,

for the anti-anginal effect:

- 10- 40 mg/day of oral 5-isosorbitmono-nitrate in combination with 1 -100 mg/kg of R- or S-enantiomer of alpha-lipoic acid.

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The general dosage range for the combinations of calcium- antagonists with R- or S-alpha-lipoic acid can be for the neuroprotective effect:

30- 90 mg /day of oral nimodipin in combination with 1-100 mg/kg of R- or S-enantiomer of alpha-lipoic acid.

for the anti-hypertension effect:

40- 360 mg/day of oral verapamil in combination with 1-100 mg/kg of R- or S-enantiomer of alpha-lipoic acid.

for the coronary-therapeutic effect:

60- 180 mg /day of oral diltiazem in combination with 1-100 mg/kg of R- or S-enantiomer of alpha-lipoic acid.

for the anti-anginal effect:

5- 15 mg /day of oral nifedipin in combination with 1-100 mg/kg of R- or S-enantiomer of alpha-lipoic acid.

for the anti-diabetic effect:

40- 240 mg /day of oral verapamil in combination with 1-100 mg/kg of R- or S-enantiomer of alpha-lipoic acid.

For instance, the preferred daily dose in the combination for calcium-antagonist, nimodipin is 90 mg orally and parenteral for nimodipin 50 mg/day intravenous and for R-alpha- lipoic acid and also for S-alpha- lipoic acid 80 mg for parenteral administration and 200 mg for the oral form.

The dosage unit of combination preparations with a calcium- antagonist, such as nimodipin in combination with the optical enantiomers of alpha-lipoic acid or a therapeutically usable salt of the same (either R-form or S-form) can contain, for instance:

a) for oral administration:

10 to 1200 mg, preferably 20 to 600 mg of optical enantiomers of alpha-lipoic acid in combination with nimodipin 0.1 to 30 mg, preferably 2 to 30 mg. The doses can be administered one to four times, preferably one to three times daily. However, a total dosage of optical enantiomers of alpha-lipoic acid should not exceed 1200 mg and for instance, of calcium- antagonist, should not exceed 360 mg per day.

b) For parenteral administration (for example, intravenous, intramuscular or intra-articular):

10 to 600 mg, preferably 15 to 500 mg of optical enantiomers of alpha- lipoic acid in the combination 1-50 mg/day intravenous, preferably 3- 40 mg/day of body weight, especially 10- 30 mg/day intravenous.

The doses can be administered for instance one to four times, preferably one to three times daily.

Obviously, even galenic preparations can be manufactured which contain two to four times the above mentioned dosage units. The following especially contain:

- tablets or capsules contain 20 to 800 mg of active substance A in combination with, for instance, a calcium- antagonist, nimodipin, 1-30 mg,

- pellets, powder or granulate contain 5 to 600 mg of active substance A in combination with the calcium-antagonist, nimodipin 1-30 mg.
- Suppositories contain 20 to 300 mg of active substance A in combination with for instance 1-30 mg of nimodipin

In case the calcium-antagonist is used in combination with R- or S-alpha- lipoic acid in the form of its salts, the halogen can be used even in excess, that means, in a higher quantity than equimolar quantity.

The general dosage range for the combinations with ACE-inhibitors with R- or S-alpha-lipoic acid can be for the cardio-cytoprotective effect:
12.5- 50 mg /day of oral captopril in combination with 1-100 mg/kg of R- or S- alpha-lipoic acid.
for the anti-hypertension effect:
1.25- 10 mg/day of oral ACE-inhibitor, ramipril, in combination with 1- 100 mg/kg of R- or S-alpha- lipoic acid.

The daily doses of the administrative forms of the combinations according to the invention for the cardio-cytoprotective and/or anti-hypertension effect contain, for

instance, preferably 1- 20 mg of lisinopril in combination with 0.1 to 600 mg, preferably 15 to 400 mg of R- or S-lipoic acid.

According to the invention, a daily dosage of combinations consisting of the above mentioned ACE-inhibitors and the optical enantiomers of alpha- lipoic acid of 5 - 50 mg of captopril, preferably 5- 25 mg and 10-600 mg of enantiomers, can be administered.

The maximum daily dosage for the cardio-cytoprotective and anti-hypertension effect should not exceed 1.2 g for the racemate or the R-or S-alpha-lipoic acid and should not exceed 50 mg for captopril.

The maximum daily dosage for the cardio-protective should not exceed 10 mg orally for the combination of ACE-inhibitor, ramipril with the R-or S-enantiomers of alpha-lipoic acid for ramipril and should not exceed 1.2 g for the enantiomers.

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The daily doses can be a single-dose administration of the total quantity or as 1- 3, especially 1- 2 partial doses per day. Generally, an administration of 1- 3 times daily is preferred.

In case solutions are used, the optical enantiomers of alpha- lipoic acid and the organic nitrates, calcium-

antagonists, ACE-inhibitors or oxyfedrin contained in the combination, are preferably used in the form of a salt.

The combination preparations according to the invention can be used even for the treatment of animals.

Generally, the oral single dose in combination lies between 2 mg/kg and 100 mg/kg of body weight for the active substance and between 0.01 and 1 mg/kg of body weight for the ACE-inhibitor for the treatment of heart insufficiency, diabetes mellitus type II, nephropathy, arteriosclerosis in horses and cattle.

The parenteral dose in the combination contains about 0.5 and 50 mg/kg of body weight of active substance A and approximately between 0.005 and 1 mg/kg of body weight of an ACE-inhibitor.

All the doses given above of the active substance A and of the organic nitrates, calcium- antagonists or ACE-inhibitors do not relate to the pharmaceutically usable salts. In case, these are used in the form of a salt, the specified doses/ dosage range are to be increased appropriately because of the higher molar weight.

The pharmaceutical combinations according to the invention in combination with organic nitrates can be used especially for the therapy and treatment of angina pectoris, left ventricular insufficiency, for treatment of

sub-acute and acute cardial pulmonary edema, pulmonary hypertension and of organic nitrate tolerance.

The indications for the combination preparations with a calcium-antagonist can be:
Diabetes mellitus, degenerative diseases of central nervous system, acute ischemic conditions, myocardial infarction, nerve degeneration (neureo-degenerative processes) cerebral neuropathy, Morbus Alzheimer, coronary insufficiency, angina pectoris, atrial fibrillation/atrial flutter with tachyarrhythmia, tachycardiac rhythm disturbances such as paroxysmal supraventricular tachycardia, Raynoud-syndrome, therapy of cardiac infarction, hypertension, hypertonic crisis, prophylaxis and therapy of ischemic neurological deficits because of cerebral vasospasm after subarachnoid hemorrhage.

The pharmaceutical combinations with ACE-inhibitors can be used especially for the treatment of hypertension, hypertonic crisis, cardiac insufficiency, diabetes mellitus type II, nephropathy, cerebrovascular events, nephropathy, cardiomyopathy and arteriosclerosis.

The pharmaceutical combination preparations with oxyfedrin can be used for prevention and treatment of angina pectoris, left ventricular insufficiency, for treatment of coronary insufficiency, partial AV- conduction

defects, subsequent states of myocardial infarction and autonomous cardial neuropathy.

The production of combination preparations according to the invention takes place in a known way, whereby the customary pharmaceutical excipients can be used. For example, those substances that are recommended or specified in the following literature as excipients for pharmaceutics, cosmetics and related fields, can be used as excipients:

Ullmanns Encyclopedia of technical Chemistry, Volume 4 (1953), Page 1 to 39;

Journal of Pharmaceutical Sciences, Volume 52 (1963), Page 918 onwards,

H. v. Gzetsch- Lindenwald, Excipients for pharmaceutics and related fields, Pharm. Ind. Book 2 (1961), Page 72 onwards.

Dr. H. P. Fiedler, Lexicon of excipients for pharmaceutics, cosmetics and related fields, Cantor KG, Aulendorf in Württemberg (1989).

The pharmaceutical and galenic handling of organic nitrates, such as glyeroltri-nitrate, isosorbit-di-nitrate or 5- isosorbitmono-nitrate and R- and/or S-alpha-lipoic acid is undertaken according to the usual standard methods.

The combinations according to the invention can be produced as follows:

200 mg of R- and/or S-alpha- lipoic acid or/and excipients are mixed well in 250 ml of aqueous isosorbitdi-nitrate or in 200 ml of 5-isosorbitmono-nitrate by stirring or homogenization (for e.g. with the help of customary mixing devices) (clear solution) whereby it is operated generally at temperatures between 20 and 50°C, preferably 20 to 40°C.

Examples for excipients are gelatins, natural sugars such as sucrose or lactose, lecithin, pectin, starches (for example, cornstarch or amyloses), cyclodextrins and cyclodextrin derivatives, dextran, polyvinyl pyrrolidone, polyvinyl acetate, acacia, alginic acid, tylose, talcum, lycopodium, silicic acid (for example, colloidal), cellulose, cellulose derivatives (for example, cellulose ether, for which the cellulose- hydroxyl groups are partially etherified with lower saturated aliphatic alcohols and/or lower saturated aliphatic oxyalcohols, for example, methyloxypropyl cellulose, methyl cellulose, hydroxypropyl- methyl cellulose, hydroxy propylmethyl-cellulose phthalate), fatty acids as well as magnesium-, calcium- or aluminum salts of fatty acids with 12 to 22 C-atoms, especially of saturated (for example, stearates), emulsifiers, oils and fats, especially vegetable (for example, peanut oil, castor oil, olive oil, sesame oil,

cottonseed oil, corn oil, wheat germ oil, sunflower seed oil, cod liver oil, even hydrated); glycerin esters and polyglycerin esters from saturated fatty acids $C_{12}H_{24}O_2$ to $C_{18}H_{36}O_2$ and their mixtures, whereby the glycerin- hydroxy groups are completely or only partially esterified (for e.g. mono-, di- and triglycerides) pharmaceutically acceptable mono- or multivalent alcohols and polyglycols such as polyethylene glycols (molecular weight range for example 300 to 1500) and also derivatives hereof, polyethylene oxide, esters of aliphatic saturated or unsaturated fatty acids (2 to 22 C-atoms, especially 10 - 18 C-atoms) with mono-valent aliphatic alcohols (1 to 20 C-atoms) or multivalent alcohols such as glycols, glycerin, diethylene glycol, pentaerythritol, sorbitol, mannite and the like, which can even be etherified, esters of citric acid with primary alcohols, acetic acid, urea, benzyl benzoate, dioxolane, glycerin formals, tetrahydrofurfuryl alcohol, polyglycol ether with C1- C12-alcohols, dimethyl acetamide, lactamide, lactate, ethyl carbonate, silicones (especially medium-viscous polydimethyl siloxanes), calcium carbonate, sodium carbonate, calcium phosphate, sodium phosphate, magnesium carbonate and the like.

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Other auxiliary materials can be substances that cause disintegration - so-called exploders- such as cross-linked polyvinyl pyrrolidone, sodium carboxy methyl starches, sodium carboxy methyl cellulose or micro-crystalline cellulose. Likewise, known coating materials can be used. Examples of such are: polymers as well as copolymers of acrylic acid and/or methacrylic acid and/or their esters; copolymers from acrylic acid esters and methacrylic acid esters with low content of ammonium groups (for e.g. Eudragit® RS), copolymers from acrylic acid esters and methacrylic acid esters and trimethyl ammonium methacrylate (for e.g. Eudragit RL); polyvinyl acetate; fats, oils, wax, fatty alcohols; hydroxypropylmethyl cellulose phthalate or -acetate succinate; cellulose- acetate phthalate- starch-acetate phthalate as well as polyvinylacetate phthalate; carboxymethyl cellulose; methyl cellulose phthalate, methyl cellulose succinate, -phthalate succinate as well as methyl cellulose- phthalic acid half esters; zein; ethyl cellulose as well as ethyl cellulose succinate; shellac, gluten; ethylcarboxyethyl cellulose; ethacrylate-maleic acid anhydride-copolymer; maleic acid anhydride-vinyl methyl ether-copolymers; styrene- maleic acid-copolymes; 2-ethyl-hexyl-acrylate maleic acid anhydride; crotonic acid-vinyl acetate-copolymer; glutaminic acid/ glutaminic acid ester

copolymer, carboxymethyl ethyl cellulose glycerine-mono-octanoate, cellulose acetate succinate and polyarginine.

Plasticizing agents for coating materials can be: Citric acid ester and tartaric acid ester (acetyltriethyl citrate, acetyltributyl, tributyl, triethyl citrate), glycerin and glycerin ester (glycerin diacetate, -triacetate, acetylated monoglycerides, castor oil), phthalic acid ester (dibutyl, diamyl, diethyl, dimethyl, dipropyl-phthalate), di-(2-methoxy- or 2-ethoxy ethyl)-phthalate, ethylphthalyl- glycolate, butylphthalylethyl glycolate and butyl glycolate; alcohols (propylene glycol, polyethylene glycol of different chain lengths), adipates (diethyl- adipate, di-(2- methoxy- or 2-ethoxy ethyl)-adipate); benzophenone; diethyl- and dibutylsebacate, dibutylsuccinate, dibutyltartrate; diethylene glycol dipropionate; ethyleneglycol diacetate, -dibutyrate, -dipropionate; tributyl phosphate, tributyrin, polyethylene glycol sorbitan monooleate (polysorbates such as polysorbate 80) and sorbitan monooleate.

For the manufacture of solutions or suspensions, water or physiologically compatible organic solvents can be used, for example

Alcohols (ethanol, propanol, isopropanol, 1,2- propylene glycol, polyglycols and their derivatives, fatty alcohols,

partial esters of glycerin), oils (for e.g., peanut oil, olive oil, sesame oil, almond oil, sunflower- oil, soyabean oil, castor oil), paraffins, dimethyl sulfoxide, triglycerides and the like.

Non-toxic parenteral- compatible diluents or solvents can be used for injectable solutions or suspensions, for example: water, 1,3-butandiol, ethanol, 1,2-propylene glycol, polyglycols mixed with water, glycerol, Ringer's solution, isotonic sodium chloride solution or even solidified oils including synthetic mono- or diglycerides or fatty acids such as oleic acid.

Known and customary solubilizers, or even emulsifiers can be used for the manufacture of preparations.

Examples of solubilizers and emulsifiers are: polyvinylpyrrolidone, sorbitan fatty acid esters such as sorbitan trioleate, phosphatide, such as lecithin, acacia, tragacanth, polyoxyethylated sorbitan monooleate and other ethoxylated fatty acid esters of sorbitan, polyoxyethylated fats, polyoxyethylated oleotriglycerides, -linolizated oleotriglycerides, polyethylene oxide-condensation products of fatty alcohols, alkyl phenols or fatty acids or even 1-methyl-3- (2-hydroxyethyl) imidazolidone- (2).

In this context, polyoxyethylated means that the relevant substances contain polyoxyethylene chains, their degree of polymerization generally lies between 2 and 40 and especially between 10 and 20.

Such polyoxyethylated substances can be obtained, for instance, by conversion of hydroxyl group-contained compounds (for instance, mono- or diglycerides or unsaturated compounds such as those that contain oleic acid radicals) with ethylene oxide (for example, 40 moles of ethylene oxide per one mole of glyceride).

Examples for oleotri-glycerides are olive oil, peanut oil, castor oil, sesame oil, cotton seed oil, corn oil.

Moreover, preservatives, stabilizers, buffer substances, flavor correcting agents, sweeteners, colorants, antioxidants and chelating agents and the like can be added.

Examples of chelating agents are:

Chelate formers such as ethylene diamino-tetra acetic acid, nitrilotri acetic acid, diethylene triamine pentaacetic acid and also their salts. Further, even those that contain active substance B in combination with, for instance, R- or S-alpha-lipoic acid in a cavity can be used as chelating agents. Examples for these are urea, thiourea, cyclodextrins, amylose. If necessary, it is possible to

stabilize the active substance molecule with physiologically compatible bases or buffers to a pH-range of about 6 to 9. Generally, as far as possible, neutral to weak basic (up to pH 8) pH-value is preferred.

Antioxidants that may be used are, for instance, sodium sulfite, sodium hydrogen sulfite, sodium metabisulfite, ascorbic acid, ascorbyl palmitate, -myristate, -stearate, gallic acid, gallic acid- alkyl ester, butyl hydroxyanisol, nordihydroguaiacic acid, tocopherols as well as synergists (substances that bind heavy metals by complex formation, for example, lecithin, ascorbic acid, phosphoric acid ethylene diaminotetra-acetic acid, citrate, tartrates). The addition of synergists considerably increases the anti-oxygenic effect of anti-oxidants.

Preservatives that may be used are, for example, sorbic acid, p-hydroxy benzoic acid ester (for example, lower alkyl ester), benzoic acid, sodium benzoate, trichlorisobutyl alcohols, phenols, cresols, benzethonium chloride, chlorohexidine and formalin derivatives.

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Examples

Example 1

Tablets with 50 mg of S- or R-alpha-lipoic acid and 8 mg of oxyfedrin

250 g of S-alpha-lipoic acid and 40 g of oxyfedrin are evenly grounded with 760 g of micro-crystalline cellulose. After sieving the mixture, 250 g of starch (starch 1500/ Colorcon), 682.5 g of lactose, 15 g of magnesium stearate and 2.5 g of highly dispersible silicon dioxide are mixed together and the mixture is pressed into tablets weighing 400.0 mg.

A tablet contains 50 mg of S-alpha- lipoic acid and 8 mg of oxyfedrin.

In the same way, tablets with 50 g of R-alpha-lipoic acid can be produced, wherein in place of 250 g of S-alpha-lipoic acid, the same quantity of R-alpha-lipoic acid is used.

If necessary, tablets can be provided with gastric juice-soluble or gastric juice permeable film coating using conventional methods.

Example 2

Capsules with 250 mg of R- or S-alpha-lipoic acid and 0.8 mg of glycerol trinitrate

250 g of R-alpha-lipoic acid are mixed with 8 g of glycerol trinitrate.

Subsequently, 1095.2 g of Miglyol®- neutral oil and 42 g of sorbitol syrup, 25 g of glycerol are then added hereto and the mixture is filled in size 00 capsules.

Miglyol® is a commercial mixture of medium-chained triglycerides.

A capsule weighing 1.42 g contains 250 mg of R- or S-alpha-lipoic acid and 0.8 mg of glycerol trinitrate.

Example 3

Suppositories with 50 mg of dihydrolipoic acid or with R- or S-alpha-lipoic acid and 30 mg of nimodipin

5 g of ascorbyl palmitate and 5 g of oxynex LM (E. Merck, Darmstadt) are suspended in 192 g of molten hard fat. Subsequently, 3 g of nimodipin and 5 g of dihydrolipoic acid are mixed together and the mixture is poured out into hollow cells of 2.3 ml after homogenization. Before sealing, the hollow cells are purged with nitrogen.

Hard fat is a mixture of mono-, di- and triglycerides of saturated fatty acids of $C_{10}H_{20}O_2$ to $C_{18}H_{36}O_2$.

Oxynex LM is a commercial additive for fats and fat-containing foodstuffs. It is a light brown, waxy mass which melts into a clear brown liquid when heated to 55°C and

contains tocopherol, ascorbyl palmitate, citric acid and lecithin.

A suppository weighing 2.1 g contains 50 mg of dihydrolipoic acid and 30 mg of nimodipin.

Suppositories with R- or S-alpha-lipoic acid can be produced in the same way, in which case, the same quantity of either R- or S-alpha-lipoic acid is used in place of dihydrolipoic acid.

Example 4

Capsules with 200 mg of dihydrolipoic acid or with R- or S-alpha-lipoic acid and 30 mg of nimodipin

200 g of R-alpha-lipoic acid are mixed with 30 mg of nimodipin.

Subsequently, 1065 g of Miglyol®- neutral oil and 100 g of sorbitol syrup, 25 g of glycerol are then added hereto and the mixture is filled in size 00 capsules. A capsule weighing 1.42 g contains 200 mg of R- or S-alpha-lipoic acid and 30 mg of nimodipin.

Miglyol® is a commercial mixture of medium-chained triglycerides.

Capsules with hydrolipoic acid or with S-alpha-lipoic acid are produced in the same way in which the same

quantity of either dihydrolipoic acid or S-alpha-lipoic acid is used instead of R-alpha-lipoic acid.

Example 5

Ampoules with 250 mg of R- or S-alpha-lipoic acid and 30 mg of calcium-antagonist, nimodipin in 10 ml

250 g of R-alpha-lipoic acid are dissolved along with 352.3 g of trometamol (2-amino (hydroxymethyl)-1,3-propandiol) in a mixture containing 8 liters of water for injection purposes and 200 g of 1,2-prophylene glycol under stirring. Subsequently, 30 g of calcium- antagonist, nimodipin, is dissolved in this preparation. The solution is filled up to 10 liters with water for injection purposes and subsequently, filtered through a membrane filter of pore width 0.2 µm with fiberglass pre-filter. The filtrate is filled up to 10 ml in sterilized 10 ml-ampoules under aseptic conditions.

An ampoule contains 250 mg of R-alpha-lipoic acid as trometamol salt and 30 mg of calcium-antagonist, nimodipin in 10 ml injection solution.

Ampoules can be produced with S-alpha-lipoic acid in the same way in which the same quantity of S-alpha-lipoic acid is used instead of R-alpha-lipoic acid.

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Example 6

Tablets with 50 mg of S- or R-alpha- lipoic acid and 30 mg of calcium-antagonist, nimodipin

250 g of S-alpha-lipoic acid and 150 g of calcium-antagonist, nimodopin are evenly grounded with 650 g of micro-crystalline cellulose. After sieving the mixture, 250 g of starch (starch 1500/ Colorcon), 682.5 g of lactose, 15 g of magnesium stearate and 2.5 g of highly dispersible silicon dioxide are mixed together and the mixture is pressed into tablets weighing 400.0 mg.

A tablet contains 50 mg of S-alpha- lipoic acid and 30 mg of calcium-antagonist, nimodipin.

In the same way, tablets with 50 mg of R-alpha-lipoic acid can be produced, wherein in place of 150 g of S-alpha-lipoic acid, the same quantity of R-alpha-lipoic acid is used.

If necessary, tablets can be provided with gastric juice-soluble or gastric juice permeable film coating using conventional methods.

Example 7

Capsules with 250 mg with R- or S-alpha-lipoic acid and 10 mg of Captopril

250 g of R-alpha- lipoic acid is mixed with 10 g Captopril.

Subsequently, 1050 g of Miglyol®- neutral oil and 85 g of sorbitol syrup, 25 g of glycerol are then added hereto and the mixture is filled in size 00 capsules.

Miglyol® is a commercial mixture of medium-chained triglycerides.

A capsule weighing 1.42 g contains 250 mg of R-alpha- or S-alpha-lipoic acid and 10 mg of Captopril.

Example 8

Tablets with 50 mg of S- or R-alpha-lipoic acid and 1.25 mg of ramipril

250 g of S-alpha-lipoic acid and 6.25 g of ramipril are evenly grounded with 792.5 g of micro-crystalline cellulose. After sieving the mixture, 250 g of starch (starch 1500/ Colorcon), 682.5 g of lactose, 15 g of magnesium stearate and 2.5 g of highly dispersible silicon dioxide are mixed together and the mixture is pressed into tablets weighing 400.0 mg.

A tablet contains 50 mg of S-alpha- lipoic acid and 1.25 mg of ramipril.

In the same way, tablets with 50 mg of R-alpha-lipoic acid can be produced, wherein in place of 250 g of S-alpha-

lipoic acid, the same quantity of R-alpha-lipoic acid is used.

If necessary, tablets can be provided with gastric juice-soluble or gastric juice permeable film coating using conventional methods.

Patent claims

1. Pharmaceutical combination preparations characterized in that they contain an alpha-lipoic acid or its metabolites and at least one organic nitrate, a calcium- antagonist, ACE- inhibitors or oxyfedrin as active substance A.
2. Pharmaceutical combination preparations according to Claim 1 characterized in that they contain alpha-lipoic acid, dihydrolipoic acid and their oxidized or reduced R- or S-enantiomers as well as metabolites of alpha- lipoic acid, such as 6,8- bisnorlipoic acid, tetranorlipoic acid as active substance A.
3. Pharmaceutical combination preparations according to Claim 1, characterized in that they contain at least one organic nitrate such as glycerol trinitrate, isosorbitdinitrate or 5-isosorbitmononitrate.
4. Pharmaceutical combination preparations according to Claim 1, characterized in that they contain at least

one calcium- antagonist of the type Verapamil, nifedipine, nimodipine, felodipine, isradipine, nitrendipine, nisoldipine, nicardipine, nivaldipine or diltiazem.

5. Pharmaceutical combination preparations according to Claim 1, characterized in that they contain at least one ACE-inhibitor of type captopril, lisinopril, perindropril-tert-butylamine, ramipril or enalapril hydrogen maleate.
6. Pharmaceutical combination preparations according to Claim 1, characterized in that they contain oxyfedrin.
7. Pharmaceutical combination preparations according to Claims 1 to 3, characterized in that 0.1 to 40 parts by weight of an organic nitrate is used for 1 to 1000 parts by weight of active substance A in the dosage unit of the combination.
8. Pharmaceutical combination preparations according to Claims 1 to 3, characterized in that the combinations contain 5-1200 mg, preferably 10- 800 mg of active substance A and 0.1 - 40 mg, preferably 0.8 -20 mg of an organic nitrate.

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9. Pharmaceutical combination preparations according to Claims 1 to 3, characterized in that the combinations

contain 1-1200 mg, preferably 2- 800 mg of active substance A and 0.1 - 40 mg, preferably 0.8 -20 mg of an organic nitrate.

10. Pharmaceutical combination preparations according to Claims 1, 2 and 4, characterized in that, 1 to 120 parts by weight of a calcium-antagonist is used for 1 to 1000 parts by weight of active substance A in the dosage unit of the combination.

11. Pharmaceutical combination preparations according to Claims 1,2 and 4, characterized in that the combinations contain 5-6000 mg, preferably 10- 3000 mg of active substance A and 5 - 120 mg, preferably 0.8 - 20 mg of a calcium antagonist.

12. Pharmaceutical combination preparations according to Claims 1,2 and 4, characterized in that the combinations contain 2-1000 mg, preferably 2- 800 mg of active substance A and 5 - 120 mg of a calcium antagonist.

13. Pharmaceutical combination preparations according to Claims 1,2 and 5, characterized in that 1 to 20 parts by weight of an ACE-inhibitor is used for 1 to 1000 parts by weight of active substance A in the dosage unit of the combination.

14. Pharmaceutical combination preparations according to Claims 1,2 and 5, characterized in that the combinations contain 5-6000 mg, preferably 10-3000 mg of active substance A and 1 - 20 mg of an ACE-inhibitor.

15. Pharmaceutical combination preparations according to Claims 1, 2 and 5, characterized in that the combinations contain 2-3000 mg, preferably 2- 1000 mg of active substance A and 1 - 20 mg of an ACE-inhibitor.

16. Pharmaceutical combination preparations according to Claims 1, 2 and 6, characterized in that, 0.1 to 40 parts by weight of oxyfedrin is used for 1 to 1000 parts by weight of active substance A in the dosage unit of the combination.

17. Pharmaceutical combination preparations according to Claims 1, 2 and 6, characterized in that the combination contains 5-1200 mg, preferably 10- 800 mg of active substance A and 4 - 48 mg of oxyfedrin.

18. Pharmaceutical combination preparations according to Claims 1, 2 and 6, characterized in that the combination contains 2-1200 mg, preferably 2- 800 mg of active substance A and 0.1 - 40 mg or especially 4-48 mg of oxyfedrin.

19. Pharmaceutical combination preparations according to Claim 1, characterized in that they contain, if necessary, additional pharmaceutical excipients and/or other additives.

20. Pharmaceutical combination preparations according to Claims 1 and 19, characterized in that they are administered in the form of tablets, capsules, pills, sugar-coated pills, aerosols, salves, creams, medical strips, suspension or solution.

21. Usage of combination preparations according to Claim 1 in combination with organic nitrates of Claim 3 for therapy and treatment of angina pectoris, left ventricular insufficiency, for treatment of sub-acute and acute cardial pulmonary edema, pulmonary hypertension and of organic nitrate tolerance.

22. Usage of combination preparations according to Claim 1 in combination with calcium-antagonist of Claim 4 for therapy of diabetes mellitus, nerve degeneration (neureo-degenerative processes) cerebral neuropathy, Morbus Alzheimer, coronary insufficiency, angina pectoris, atrial fibrillation/atrial flutter with tachyarrhythmia, tachycardiac rhythm disturbances such as paroxysmal supraventricular tachycardia, Raynoud-syndrome, therapy of cardiac infarction,

hypertension, hypertonic crisis, prophylaxis and therapy of ischemic neurological deficits because of cerebral vasospasm after subarachnoid hemorrhage.

23. Usage of combination preparations according to Claim 1 in combination with ACE-inhibitors of Claim 5 for treatment of essential hypertension, cardiac insufficiency, hypertension, hypertensive cardiomyopathy, myocardial insufficiency, diabetes mellitus type II, nephropathy, arteriosclerosis, nephropathy and cerebrovascular events.

24. Usage of combination preparations according to Claim 1 in combination with oxyfedrin for prevention and treatment of angina pectoris, left ventricular insufficiency, subsequent states after myocardial infarction, partial AV- conduction defects.

25. Method for manufacture of combination preparations according to Claim 1 characterized in that the active substance A and at least one organic nitrate, calcium- antagonist, ACE-inhibitor or oxyfedrin or their pharmaceutically usable salts are mixed or homogenized with pharmaceutical excipients and/or other additives at temperatures between 0 and 120°C, preferably 20 to 80°C, and the mixture so obtained is filled in hollow cells of appropriate size

or in capsules or granulated and then, if necessary, pressed into tablets by adding other customary auxiliary substances or filled in capsules.

26. Method for manufacture of combination preparations according to Claim 1 characterized in that the active substance A and at least one organic nitrate, calcium- antagonist, ACE-inhibitor or oxyfedrin or their pharmaceutically usable salts are dissolved in water, physiologically harmless alcohols, oils or dimethyl sulfoxide or mixtures thereof at temperatures between 20 and 100°C, if necessary, in the presence of chelating agent and/or of emulsifier, and the solutions and suspensions so obtained are filled up.

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27. Pharmaceutical combination preparations according to Claim 1, characterized in that the active substance A and at least one organic nitrate, calcium-antagonist, ACE-inhibitor or oxyfedrin exist in the same dosage form for administration at the same time.

28. Pharmaceutical combination preparations according to Claim 1, characterized in that the active substances A and B exist in different dosage forms for administration at the same time.